NITROGEN FERTILIZATION AND SUPPLEMENTATION EFFECTS ON LIMPOGRASS CHEMICAL CHARACTERISTICS AND UTILIZATION BY CATTLE

By

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A DISSERTATION PRESENTED TO THE GRADUATE SCHOOL
OF THE UNIVERSITY OF FLORIDA IN PARTIAL FULFILLMENT
OF THE REQUIREMENTS FOR THE DEGREE OF
DOCTOR OF PHILOSOPHY

UNIVERSITY OF FLORIDA

1995

I believe a leaf of grass is no less than the journey-
work of the stars,
And the pismire is equally perfect, and a grain
of sand, and the egg of the wren,
And the tree-toad is a chef-d'oeuvre for the highest,
And the running blackberry would adorn the
parlors of heaven,
And the narrowest hinge in my hand puts to scorn
all machinery,
And the cow crunching with depress'd head
surpasses any statue,
And a mouse is miracle enough to stagger sextillions
of infidels.

SONG OF MYSELF
(Walt Whitman)

To my father (in memory) who taught me about faith through his love for Nature and his conviction of the power of working the land to create a less unfair world for the modest people.

To Jacy, Susana, Manuela, Daniel, and Tiago.

ACKNOWLEDGMENTS

The author expresses grateful appreciation to Dr. Lynn E. Sollenberger, committee chairman, for his guidance, support, encouragement, friendship, and for his many contributions to the preparation of this manuscript. Appreciation is also extended to committee members Drs. John E. Moore, William E. Kunkle, Joseph H. Conrad, and Carrol G. Chambliss for their guidance, support, criticism, teaching, and professional assistance throughout the graduate program and for reviewing this manuscript.

The author would also like to acknowledge the EMBRAPA (Empresa Brasileira de Pesquisa Agropecuária), which provided financial support, and the EMPARN (Empresa de Pesquisa Agropecuária do Rio Grande do Norte), which authorized his participation in this graduate program.

Thanks are extended to Dr. William D. Pitman, former member of the supervisory committee, and to Dr. K.H. Quesenberry for participating in the qualifying exam and for his assistance as former graduate coordinator. The author is especially grateful to Dr. J.M. Bennett, Chairman of the Agronomy Department, for his receptiveness and for providing the opportunity to study at the University of Florida.

Special gratitude is expressed to the Animal Science Department for cooperation and assistance, particularly to Dr. D.B. Bates for the generous use of his laboratory. The author thanks Dr. Ramon C. Littell and Mr. Jay Harrison for statistical support, and Dr. A.C. Hammond for his help with the plasma urea nitrogen analyses. Thanks are due to Dr. Glen A. Broderick and Mr. Bradford Ricker at the U.S. Dairy Forage Research Center, Madison, WI, for continuous support and discussion and for some protein degradation analyses. The author also wishes to thank Kay Richardson for providing the animals for the grazing trial.

Special thanks are also due to members of the staff at the University of Florida who helped in conducting this research. They include Richard Fethiere, John Funk, Nancy Wilkinson, Pam Miles, Sid Jones, Dwight Thomas, Al Boning, Nancy Carter, Mel Tooker, Jeanette Filer and Angelita Mariano. To fellow graduate students Robert Spitaleri, Carlos Pedreira, Pedro Yepes, Edison Pott, Juan Velasquez, Bruce Mathews, Delroy McDonald, Elide Valencia, Juli-Anni Royes, Francisco Olbrich, and visiting professor Dr. Ivo Andrade, the author expresses his gratitude for their friendship, assistance, and presence during the pleasant and difficult moments during the program. To Dr. Clarence B. Ammerman, the author expresses thanks for his kindness and cooperation which provided a pleasant work environment in the Animal Nutrition Laboratory.

Finally, to his wife Susana, daughter Manuela, and sons Daniel and Tiago, the author is grateful for their love, patience, and encouragement during the whole program.

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By

Guilherme Ferreira da Costa Lima

August 1995

Chairman: Lynn E. Sollenberger
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Floralta limpograss (*Hemarthria altissima* [Poir.] Stapf. et C.E. Hubb.) is growing on an estimated 50,000 ha in Florida. It is well documented, however, that low N concentrations of Floralta can limit intake and gains of growing animals grazing during summer. The main objective of this research was to evaluate management alternatives for raising beef replacement heifers and steers on limpograss pastures in Florida. Two principal studies are described. The first was conducted during the summers of 1992 and 1993 and tested effects of pasture N fertilization (50 and 150 kg ha^{-1}) and protein supplementation (NONE, corn + urea [CU], and corn + urea + rumen undegradable protein [CUUP]) on gains of yearling heifers (*Bos* spp.). The second study used in-vitro analysis to assess protein degradability of limpograss and two other warm-season grasses grown during 1994 at different N fertilization

rates, seasons and maturities.

Heifer average daily gain (ADG) increased greatly when protein supplements were fed to animals grazing limpograss fertilized with 50 kg N ha^{-1}, but responses to supplementation were reduced when pastures received 150 kg N ha^{-1}. Increasing N fertilization from 50 to 150 kg ha^{-1} increased ADG and gain per hectare (GAIN). Heifers receiving the CUUP supplement had a better overall ADG and GAIN than those receiving CU or no supplement. Analysis of cost per kg of additional gain (above 50NONE) suggested advantages for the low N rate-CUUP ($0.41/kg) and high N rate-NONE ($0.39/kg) treatments.

In the second study Tifton-85 bermudagrass (*Cynodon dactylon* L.) and Pensacola bahiagrass (*Paspalum notatum* Flugge) had the highest N concentrations and Floralta the lowest. Neutral detergent insoluble N (NDIN) composed almost half of total N of these grasses. A significant portion of NDIN was acid detergent insoluble N indicating reduced availability of an already limited nutrient. In-vitro N degradability was lower than values reported in the literature. Lower N degradability and high N association with the cell wall indicates a high proportion of slowly degradable protein in these grasses. In conclusion, low N concentration and slow degradability of N in limpograss cause N deficiencies of grazing cattle. Nitrogen deficiencies can be overcome by pasture N fertilization or by providing N supplements to the cattle.

CHAPTER 1
INTRODUCTION

Beef cattle production systems throughout the tropics and subtropics are based on perennial grass pastures. Tothill (1978) suggested that to appreciate the fundamental relationships that occur in pastures, it is essential to understand the origin and evolution of the grasses upon which the system is based. Most tropical grasses originated from Africa where they evolved under selection pressure in the form of animal grazing. In this environment, tropical grasses developed mechanisms that confer persistence under grazing, a development which led Renvoise and Clayton (1992) to describe the relationship between grass and grazing animal as 'quasi-symbiotic'. In contrast, most tropical legumes evolved in the absence of grazing in the American tropics. In light of these differences in evolutionary history, it is not surprising that grasses are better adapted than legumes to extensive, low input systems of the tropics. Unfortunately, persistent tropical grasses are often low in nutritive value and cannot always supply enough energy and protein to grazing ruminants. Numerous approaches have been tried to address this problem, including planting of legumes, pasture fertilization, and supplementation of grazing animals.

A tropical grass that originated in Africa is 'Floralta' limpograss (*Hemarthria altissima* [Poir.] Stapf et C. E. Hubb.). Floralta was released officially as a cultivar in Florida in 1984 (Quesenberry et al., 1984), and is currently growing on an

estimated 50,000 ha in the state (Quesenberry, 1993). Excellent cool-season growth, high dry matter (DM) production, and high in vitro digestibility have made Floralta limpograss attractive to beef producers with large holdings of poorly drained soils. Despite its high in vitro digestibility, a series of animal performance trials (Rusland et al., 1988; Sollenberger et al., 1988, 1989; and Holderbaum et al., 1991) definitively established that cattle grazing limpograss pastures gain at a slower rate than expected and the primary reason for this response appears to be low limpograss N concentration.

The main objective of this research was to evaluate management alternatives for raising beef replacement heifers on limpograss pastures in Florida. Nitrogen availability and utilization were regarded as the main constraints to productivity of the system, and N fertilization and protein supplementation (degradable and undegradable) were considered to be the most practical management practices to optimize animal performance. Two principal studies are described. The first study evaluates the effects of pasture fertilization with N and supplementation of cattle diets with N on replacement heifer and pasture performance. The second study uses in-vitro analysis to assess protein degradability of limpograss and two other tropical grasses at different ages, N fertilization rates, and seasons. Data from the second study will aid in explaining responses observed in the animal performance trial.

CHAPTER 2
LITERATURE REVIEW

Beef-Forage Systems in the Tropics and Subtropics

Greater use of concentrate feeds and other supplements during the last half century have markedly increased ruminant animal production in many areas of the world. In a large proportion of tropical and subtropical regions, this approach has not been economical, and animal production continues to be based on native grasslands or planted pastures.

Hodgson (1990) indicated that approximately 25% of the total land area of the world is classified as grazing land. Many of these areas are unsuited for crop production because of soil, slope, or other environmental limitations. For example, in tropical America, infertile and highly acid Oxisols and Ultisols occupy about 850 million ha, or about one-half of tropical America's land surface (Cochrane, 1979). Utilization of grasslands by ruminants is an important means of producing high quality human food on land unsuited for crop production (Leaver and Weissbach, 1993). Hodgson (1990) describes grazing as a way of gathering and concentrating nutrients from extensive areas of land that otherwise could not be exploited.

The challenge to researchers studying the forage-livestock system in the tropics and subtropics is to develop efficient ruminant production systems in unfavorable

environments and when inputs are limited. Lazenby (1988) suggested that grasses are ideal components of these systems because of their ability to withstand grazing and fire and to survive periods of drought and extreme heat and cold. Indeed, based on research and extension efforts of national and international organizations, millions of hectares have been planted to grass species adapted to low-input systems, including *Brachiaria decumbens* and *Andropogon gayanus* (Toledo and Nores, 1986). In contrast, tropical legumes continue to be used on a small scale because of susceptibility to pests and diseases, inability to compete with vigorous tropical grasses, poor persistence under grazing, and greater management and input requirements.

Despite many advances, technology transfer leading to adoption by farmers has not been as successful as hoped in many subtropical and tropical regions. Factors contributing to this problem include land tenure, available credit, low salaries, weak extension service, low risk tolerance by farmers, malnutrition, and other prevailing social and economic conditions. For the lowest income quartile of the population in tropical Latin America, Toledo and Nores (1986) reported beef expenditure shares ranged from 12.4 to 26% of total household income. Compared with the animal protein consumption in wealthy countries of 75 g per person per day, amounts in the poorest nations were 5 g (Mannetje, 1982). Efficient low-input grassland systems can make an important contribution to improving human nutrition in developing countries. These needs are becoming increasingly critical because over 80 million people are added to the global population each year (Mooney, 1993).

An important component of efficiently managed forage-livestock systems is the linkage of cattle and forage production cycles. Sollenberger and Chambliss (1991) explained that matching plant and animal cycles maximizes efficiency of forage utilization and minimizes the need to harvest forage or purchase feeds to fill gaps. Comparing the relative costs of grazed grass, conserved forages, and purchased feeds per unit of metabolizable energy (ME), Leaver (1988) suggested a ratio of 1:2:4. In high forage systems in New Zealand, 90% of the total nutrients fed come directly from grazing (Hodgson, 1990). Even in the United States where highly intensive livestock systems are widespread, low input grass systems have a place. A good example is 'Pensacola' bahiagrass (*Paspalum notatum* Flügge) pastures that are the base for the beef cattle industry in peninsular Florida. As pointed out by Pitman et al. (1992), bahiagrass withstands overgrazing and infrequent fertilization, characteristics of the low level of management often provided. These authors implied that with minimal fertilizer inputs, bahiagrass can be considered a reliable and sustainable pasture grass for the economic conditions prevailing in the region.

Unquestionably, beef production in the tropics is still far from its potential. Jarrige and Auriol (1992) revealed that 80% of the world's beef is produced in temperate zones with less than 45% of the total cattle population. Severe limitations to tropical pasture production are the pronounced seasonality of herbage growth (Reid and Klopfenstein, 1983) and low nutritive value during most of the period of active pasture growth (Mannetje, 1982). In surveys of literature, Norton (1982) and Minson (1990) concluded that only half of the tropical grass samples would meet the crude

protein (CP) requirements for cattle maintenance. Concentrations of N below 10 g kg^{-1} of DM affect the fermentative efficiency of the rumen bacteria and reduce herbage intake and digestion (Hodgson, 1990).

Comparisons of DM production and nutritional characteristics of tropical and temperate forages provide a basis for understanding differences in animal performance from them. Potential forage dry matter yields from tropical grasses are much higher than temperate ones and can reach 40 to 50 Mg ha^{-1} yr^{-1} (Humphreys, 1991). However, on-farm yield is less than one-third this amount, and high structural fiber and low protein concentrations of the grasses severely restrict animal production (Minson et al., 1993). Cowan et al. (1993) showed that feeding systems using tropical grass pastures have a milk production per cow below 12 kg d^{-1} or 3,600 kg per lactation. Gains of steers grazing N-fertilized warm-season grasses (WSG) generally do not exceed 0.6 kg d^{-1} over an entire grazing season (Mott and Moore, 1977). By comparison, Holmes (1989) lists characteristics of higher quality grass forages in Great Britain. Their potential yield of DM ranges from 2 to 25 Mg ha^{-1}, metabolizable energy concentration from 6 to 13 MJ kg^{-1} DM, milk yield per cow of 30 kg d^{-1}, and daily gains of 1.25 kg on cattle and 400 g on sheep are achievable on pastures.

It is not appropriate to judge forage management systems only in terms of their outputs. Sometimes the available resources are so scarce and the level of affordable risk by the farmers so small that low-input, extensively managed systems are the only profitable alternative. As stated by Nix (1989), the economics of grass management

systems will depend on optimal level of target yield and level of inputs. Even taking into account the low level of inputs affordable by tropical grass-beef cattle systems, some management alternatives may provide economic responses. One of the proposed areas of focus of this dissertation is to evaluate the utilization of N fertilization and protein supplementation as tools to optimize animal performance in grazing systems.

Floralta Limpograss

The history of Floralta limpograss in Florida already has filled several chapters. This literature describes the research that lead to the release of several limpograss cultivars. The first lines of limpograss to be released as cultivars in Florida were Redalta, Greenalta, and Bigalta in 1978 (Quesenberry et al., 1978). Floralta was released in 1984 (Quesenberry et al., 1984), and currently Floralta and Bigalta are planted on an estimated 50,000 ha in Florida (Quesenberry, 1993). Limpograss is native to Southern Africa and was introduced in Florida in 1964 from plant collections made by the USDA Plant Introduction Service (Oakes, 1973). During the three decades since those early collections, a consistent program of limpograss evaluation has been ongoing at the University of Florida. In this process, feedback from ranchers has guided establishment of research priorities. This section of the literature review will summarize the steps leading to release of Floralta and describe its contribution to the Florida beef cow-calf system.

Initially, several trials (Hodges and Martin, 1975; Ruelke et al., 1976; Oakes, 1978; Quesenberry et al. 1978; Ruelke, 1978; Christiansen, 1982) were conducted in

greenhouses or small plots to compare limpograss lines with commonly used grasses such as Pensacola bahiagrass and bermudagrasses (*Cynodon dactylon*). Several positive attributes of Floralta emerged from this preliminary screening. Among those are aggressive growth, good adaptation to poorly drained soils, high DM production, high digestibility, and good cool-season growth.

Floralta had superior total DM accumulation compared to Bigalta and Redalta (Ruelke, 1978; Quesenberry et al., 1984). Ruelke and Quesenberry (1983) managed Floralta for late fall utilization and obtained a yield plateau of 10.0 Mg DM ha^{-1} by about the first of October. Kalmbacher et al. (1987) and Adjei et al. (1989) reported annual average DM yield for Floralta of close to 17.0 Mg ha^{-1}.

In addition to high DM yields, limpograss cultivars also had in vitro digestible organic matter (IVDOM) concentrations higher than many tropical grasses (Carvalho, 1976; Moore et al., 1981). Among limpograss lines, IVDOM of Floralta (606 g kg^{-1}) was superior to Redalta (545 g kg^{-1}) and Greenalta (586 g kg^{-1}) and comparable to Bigalta (605 g kg^{-1}) (Ruelke et al., 1976). Screening 639 samples of grasses submitted to the Florida Extension Forage Testing Program, Moore et al. (1991) reported that 68% of limpograss samples had more than 51% total digestible nutrients (TDN). In contrast, only 11% of bahiagrass samples had more than 51% TDN. Kalmbacher et al. (1987) monitored limpograss IVDOM during the year and described variations according to season. Values were 477 g kg^{-1} in early spring, 628 g kg^{-1} in early summer, 533 g kg^{-1} in late fall, and 503 g kg^{-1} after the first frost in December.

The tetraploid cultivars of limpograss, Floralta and Bigalta, generally have higher IVDOM than the diploids. These differences were associated with a lower percentage of vascular bundles in the stem cross-sectional area of the tetraploids compared with the diploids (Schank et al., 1973; Ruelke, 1978).

What distinguished Floralta from Bigalta in the evaluation process was the better persistence under grazing of Floralta. The markedly superior persistence of Floralta was manifested clearly in some mob grazing experiments (Mislevy et al., 1984; Quesenberry et al., 1984). Floralta maintained a better percent ground cover (75 to 95) compared with Bigalta (5 to 85) (Ocumpaugh, 1982), and Bigalta stands deteriorated rapidly, especially when subjected to higher stocking rates (Pitman et al., 1994). Evaluating limpograss persistence, Christiansen et al. (1981) related frequent cutting with decreasing energy reserves of Bigalta, but not in Floralta. The authors implied that this difference in persistence could be related to reserve carbohydrate status. Sollenberger et al. (1989) suggested that Floralta provides the best combination of persistence under grazing and high IVDOM of the four limpograss cultivars released in Florida.

The positive agronomic and nutritive value characteristics of Floralta limpograss indicated that animal performance tests were warranted. Results of these studies were surprising, however (Euclides, 1985; Sollenberger, 1985). Average daily gains (ADG) were similar on Floralta (0.35 kg d^{-1}) and Pensacola bahiagrass (0.33 kg d^{-1}) in the first year of a continuous stocking trial at the Beef research Unit (BRU) at Gainesville, Florida (Quesenberry et al., 1984). Gain per hectare was twice

as great for Floralta because of its higher carrying capacity (three animals per hectare for bahiagrass versus four for Floralta) and longer grazing season. In the following 2 yr, ADGs on Floralta were similar to but those on bahiagrass. Experienced ranchers in Florida (Williamson, 1993) reported an increase in stocking rate of 80 to 100% after converting bahiagrass pastures to Floralta. Sollenberger et al. (1988) stated that on continuously stocked pastures utilized at moderate grazing pressure, animal performance on Floralta is not likely to surpass that on bahiagrass. Using a different approach, Sollenberger et al. (1989) reported greater gain per hectare and higher levels of stocking for rotationally stocked Floralta than bahiagrass, but ADG did not differ for the two grasses.

It has been suggested that the low ADG of steers and heifers grazing limpograss during midsummer to late fall in Florida is due to a deficiency of dietary protein (Christiansen, 1982; Euclides, 1985; Sollenberger, 1985). Crude protein concentrations lower than 50 g kg^{-1} DM were described by Euclides (1985) during large portions of the grazing season. Analyzing 69 samples of limpograss hay submitted by Florida producers, Moore (1992) reported unusually low CP concentration (78.5% of the samples had CP $<$ 70 g kg^{-1}). Comparing continuously stocked Pensacola and Floralta, Sollenberger et al. (1988) found CP of esophageal extrusa of steers grazing limpograss to be 35 g kg^{-1} lower than bahiagrass (58 vs. 93 g kg^{-1}). They concluded that at these CP concentrations, intake and gains on limpograss likely were limited by a protein deficiency during parts of the grazing season.

A series of animal performance trials (Rusland et al., 1988; Sollenberger et al., 1988, 1989; and Holderbaum et al., 1991) definitively indicated a marked influence of limpograss N concentration on summer performance of grazing animals in Florida (summer slump). During late July to mid-September, cattle grazing many WSG pastures in Florida (particularly limpograss and bahiagrass) lose weight or gain at a slower rate than during spring, early summer, and fall.

In addition to the low N concentration of Floralta, N availability has been indicated as a potential problem. Studying CP concentration of the whole canopy of Floralta, Holderbaum (1989) reported that a high proportion of plant N was associated with the cell wall fraction. Neutral detergent fiber (NDF) N represented 60 to 70% of limpograss total N, with 22 to 34% associated with the indigestible acid detergent fraction.

Another important consideration in the overall evaluation of limpograss is its unusual ratio between DOM and CP (DOM/CP ratio). According to Moore and Kunkle (1995), when DOM/CP ratios are low (<7), it is considered that there is a balance between DOM and CP with adequate protein to match the energy in the forage. High DOM/CP (>7) indicates a deficiency of protein relative to energy. Of 69 limpograss samples analyzed, Moore et al. (1991) identified 81% with TDN/CP ratios above 8 (23% above 13). A plasma urea N (PUN) concentration analysis was used by Hammond et al. (1993) as an indicator of the protein to energy ratio in the diet and to predict response to protein or energy supplementation in grazing cattle. They reviewed literature of management studies with steers and heifers grazing

limpograss and bahiagrass in Florida, and found that a higher response to protein supplementation occurred when PUN concentration was below 9 mg dL^{-1}.

This information is very important in understanding how management alternatives can address protein deficiencies and improve animal performance on grazed limpograss pastures. Several management alternatives have been proposed in order to address CP limitations and balance the DOM/CP ratios of Floralta limpograss. These include rotational stocking plus N fertilization (Sollenberger et al., 1989), overseeding limpograss pastures with legumes like aeschynomene (*Aeschynomene americana* L.) (Sollenberger et al., 1987a, Rusland et al., 1988, Holderbaum et al., 1991; Chaparro et al., 1992), non-protein N (NPN) supplementation (Holderbaum et al., 1991), and hay ammoniation (Brown, 1991). The results of some of these experiments will be discussed in the following sections of the literature review about N fertilization and supplementation.

Nitrogen Utilization by Ruminants Grazing Warm-Season Grass Pastures

In the particular case of limpograss and also for some other WSG, N is the first limiting nutrient to the performance of grazing ruminants. In these situations, Nolan and Leng (1989) recognized the need to understand the range of management options to provide more N. To employ proper management it is necessary to understand how grazing cattle utilize herbage N. Because N functions are so essential for rumen and tissue metabolism, several different approaches can be chosen to optimize efficiency of N utilization. Special emphasis will be given in this section of

the literature review to particular characteristics of protein degradation, energy-protein balance, and to how microbial and host protein requirements can influence the efficiency of protein utilization by grazing ruminants.

Broderick (1994) proposed that any method to assess protein quality of the forage needs to compare bacterial crude protein (BCP) and undegradable intake protein (UIP) provided by the forage and required by the animals. The development of the National Research Council (NRC) system (1985) for ruminant nitrogen usage was an important step toward a better understanding of protein fractionation and utilization by ruminants. It enhanced the discussion of dynamics of rumen fermentation and potential loss of N as ammonia. It also was one of the first systems to establish dietary intake protein and ruminant requirements in terms of rumen degradable and undegradable N. Taking into account the chemical and kinetic criteria, Van Soest (1982) recognized four classes of feed N including soluble NPN, rapidly degraded protein, more slowly degraded protein, and unavailable N. Estimations on protein degradabilities for supplements have been published in several reviews. However, information on grass and legume protein degradabilities are almost nonexistent (Hart and Leibholz, 1990; Beever, 1993a). Studying strategies for predicting the first limiting nutrient for grazing cattle, Klopfenstein (1993) selected grass protein composition as the largest unknown. Data available on undegraded dietary protein (UDP) of fresh grass were in the range of 260 to 290 g kg^{-1} of CP intake (Van Straalen and Tamminga, 1990; Merchen and Bourquin, 1994). Protein of WSG is generally thought of as being more slowly degraded than that of cool-season

grasses (Akin, 1989), but this may provide a greater proportion for utilization in the small intestine (Karges et al., 1992). Faichney (1983) labelled the protein reaching the duodenum as UDP, microbial protein, and endogenous protein. Under most circumstances, forage protein is quite extensively degraded by ruminal microorganisms (Beever, 1993a; Mercher and Bourquin, 1994). This extensive rumen proteolysis of fresh forages increases the dependency of the grazing host on microbial protein as a source of amino acid production. The NPN component in fresh forages may approach 40 to 50% of the total protein (particularly if recently fertilized), comprising free amino acids, amides, and nitrates (Beever, 1993b).

Ruminal energy to protein balance is another essential requirement for efficient N use. There is a need to synchronize available energy and N to the rumen biota (Van Vuuren et al., 1990). Nolan and Leng (1989) indicated that normally the energy/protein ratio is the primary limitation to voluntary feed intake and to efficient use of absorbed nutrients, particularly for WSG. Mature tropical grasses frequently have ratios of TDN/CP greater than 20 (Hogan, 1982). He suggested that ratios of 10:1 will provide ammonia levels of 0.02 g L^{-1} of N or less which is a minimal level to maintain rumen function. Nitrogen intake can directly affect energy utilization and voluntary intake through its influence on rumen microorganisms and hence on rate of substrate degradation (Ørskov, 1976). The establishment of an optimal energy/protein ratio is complicated because two requirements must be met, one for the rumen microbes and another for the host animal (Clark and Davis, 1983; Oldham, 1984). This synchronization can be achieved by combining feed components that will

optimize digestion in the rumen, microbial protein synthesis, passage of the nutrients to the absorption sites, and milk production (Clark, 1993). The protein value of a diet is described by Hogan (1982) as an expression of the capacity of the diet to supply adequate ammonia and essential and non-essential amino acids to support protein synthesis at the rate permitted by the energy supply.

To understand utilization of protein from forages it is essential to determine the net outcome of N transactions in the rumen (Thomas and Chamberlain, 1990). Ruminants need N in two principal forms, as ammonia for the rumen microflora, and as dietary preformed amino acids absorbed in the intestines. Ammonia is essential as the main N source for the majority of rumen bacteria (Akin, 1982) particularly for cellulolytic strains. However, for forage-fed ruminants, 30-80% of microbial protein synthesized by mixed bacteria is apparently derived from peptides and amino acids (Nolan and Leng, 1989). Owens et al. (1991) concluded that when the amount of N available for ruminal microbes is below 26 g kg^{-1} of organic matter (OM) digested, ammonia supply is inadequate and bacterial CP yield will be depressed. Nolan (1993) associated the microbial growth efficiency with quantity and quality of the diet, mix of microbial species present, substrates, and ruminal conditions such as partial pressure of H, pH, and turnover time.

The strategic importance of microbial protein synthesis for grazing ruminants is emphasized by several authors (Van Vuuren et al., 1990; Broderick et al., 1991; Beever 1993a, 1993b; Merchen and Bourquin, 1994). They indicated that the proportion of microbial N in total abomasal or duodenal N is almost two-thirds (range

47 to 81%) in animals fed forage-based diets. Microbial protein is considered a high quality N source with a well-balanced amino acid profile (Merchen and Titgemeyer, 1992). The typical composition of rumen microbial cells is 32% true protein, 10% small molecules (mainly N compounds), 8% nucleic acids, 11% lipids, 9% cell wall, 17% polysaccharide, and 13% ash (Nolan and Leng, 1989). Microbial protein synthesis averages 32 g N kg^{-1} of OM apparently digested in the rumen (ARC, 1984; Merchen and Bourquin, 1994). Different ranges are published by Beever (1993a) (30 to 46 g kg^{-1}), Merchen and Bourquin (1994) (17 to 71 g kg^{-1}), and other lower values suggested for grass diets (23 to 25 g kg^{-1}) (Hart and Leibholz, 1990; Van Vuuren et al., 1990). Efficiency of amino acid utilization is assumed to be 0.80 (ARC, 1984) of the amino acids apparently absorbed, but there is increasing evidence of much lower values for forage diets. When fresh forages are fed, it is common for forage protein to be very degradable in the rumen. As a result, there may be imbalances of N and energy supply, because the requirement for ammonia may be exceeded by several times (particularly in fertilized grasses and legumes). According to Beever (1993b), this high load of ammonia from forage diets such as in high N containing legumes may adversely affect the consequent hepatic output of amino acids. Symonds et al. (1981) reported cows being able to remove up to 12 g NH_3-N h^{-1} by hepatic conversion to urea, with severe clinical changes observed above that level. This conversion also has its cost in energy for the animal; Tyrrel and Moe (1985) cited by Van Soest (1982) suggested 12 kcal g^{-1} N.

As was described in this portion of the review, efficiency of protein utilization by ruminants grazing WSG is a complex subject which comprises several interconnected factors. In order to better understand factor interactions and outputs, Broderick (1994) suggested the necessity of quantifying ruminal degradation and passage of forage protein, intestinal digestibility of the UIP, and rumen fermentation of the energy source.

The following section of the review will address characteristics of WSG that are constraints to the efficiency of N utilization, such as low N concentration, high CP degradability in the rumen, high fiber (structural carbohydrates), and low available energy from soluble carbohydrates. Nitrogen fertilization and supplementation will be the potential management alternatives to overcome these constraints that will receive the greatest attention.

Effect of N Fertilization on Grass Growth, Nutritive Value, and Utilization

Nitrogen fertilization has been used worldwide as a powerful management tool to improve cattle performance on grass-based systems. One advantage of this practice is its ability to reduce the seasonal disparities between herbage production and animal requirements (Hodgson, 1990). Other beneficial aspects of N application include the increase in herbage N concentration (Dougherty and Rhykerd, 1985; Van Soest, 1985; Hodgson, 1990; Nolan, 1993), increase in DM accumulation (Humphreys, 1987; Hodgson, 1990; Leaver and Weissbach, 1993), increase in output of milk and liveweight gain (Hodgson, 1990; Teitzel, 1991), and increase in stocking rate and

gross margin per hectare (Nix, 1989; Leaver and Weissbach, 1993). On the other hand, there have been many concerns raised related to low efficiency of N use by ruminants (Dougherty and Rhykerd, 1985; Van Der Meer and Van Lohuyzen, 1986; Deenem and Lantinga, 1993; Leaver and Weissbach, 1993) and potential for contamination of the environment (Humphreys, 1987; Scholefield et al., 1988; Leaver and Weissbach, 1993).

Nitrogen in soil exists primarily as inorganic N (NO_3^- and NH_4^+) and organic matter N, and plant roots absorb N from soil solution in inorganic forms (Follet and Wilkinson, 1985). Nitrate is the most abundant form of N taken up by plants (Salisbury and Ross, 1985). Soil NO_3^- levels are extremely variable depending on soil type, climate, season, and man's activities, whereas NH_4^+ levels tend to be constantly low (Russel, 1973).

Nitrate taken up by the plant must be reduced to NH_3 before it can be combined with carbon skeletons to form amino acids. Dougherty and Rhykerd (1985) described the chloroplast of leaves and stems of plants as the most important centers for nitrate reduction and amino acid synthesis due to large availability of energy and reducing power.

Van Soest (1982) divided total plant N in about 60 to 80% true protein (70 to 90% according to Tamminga, 1986), with the remainder made up by soluble NPN and a small amount of lignified N. He also listed the components of the soluble NPN fraction of fresh forages as nitrate, non-essential amino acids, glutamine, asparagine,

and γ-amino-butyric acid. Dougherty and Rhykerd (1985) listed nitrate, amino acids, and amides as the main forms of N found in the sap of xylem and phloem.

Nitrogen fertilization of grasses increases CP concentration, depresses soluble carbohydrates in leaves and stems, and promotes more rapid lignification (Van Soest, 1985). If climatic conditions are favorable, the application of N fertilizer normally stimulates tiller development, increases leaf size, increases water concentration, decreases soluble carbohydrate concentration, and increases yield and carrying capacity (Nelson and Moser, 1994; Buxton and Fales, 1994). Under N stress, Bushby et al. (1992) hypothesized that plants maximize soil exploration with large allocation of resources below ground causing constraints on yields from tops. Tropical grasses usually respond to added N in the order of 20 to 50 kg DM kg^{-1} of applied N (Humphreys, 1987; Hodgson, 1990). Studying tropical grasses in Queensland, Cook and Mulder (1984) reported that every additional kg of N provided 0.5 to 1.0 kg CP ha^{-1} in the period of low productivity and 3.0 to 4.5 kg CP ha^{-1} in the more favorable periods.

Reviewing more than 80 references about N fertilization and digestibility, Wilson (1982) found an almost equal distribution of positive, nil, or negative effects of N on dry matter digestibility (DMD) of grasses. Messman et al. (1991) reported little or no effect on extent of fiber digestion due to N fertilization. On the other hand, George and Hall (1983), cited by Buxton and Fales (1994), studying N fertilization of WSG with low N concentration, reported increases in rates of NDF digestion and intake by ruminants. The influence of N fertilization on grass intake is

controversial. Some authors (Minson and Milford, 1967; Puoli et al., 1991) reported positive responses for sheep and cattle, but others (Horn et al., 1979; Decker et al., 1971; quoted by Paterson et al., 1994) did not find any increase in intake. A reason for lack of response of intake to N fertilizer is explained by Deinum and Dirven (1976) and Wilson and Mannetje (1978) as being due to an increase in leaf senescence and to production of larger stems. Higher intake of N-fertilized forages has been associated with increased succulence, higher concentration of digestible energy, lower cell wall concentration, increases in leaf:stem ratio, and lower incidence of foliar diseases (Dougherty and Rhykerd, 1985). However, they suggested that an increase in forage availability may be the major factor influencing intake. Minson (1973) stated that intake responses due to N fertilization only happen when N concentration level is below 10 g kg^{-1}, i.e., N is deficient.

The efficiency of fertilizer N use is defined by Deenen and Lantinga (1993) in two ways. One way is apparent recovery of N, which expresses the increase in the amount of N contained in the harvested herbage as a percentage of that applied in fertilizer. Secondly, efficiency is described as the amount of harvested herbage per kg of N applied in fertilizer.

Dougherty and Rhykerd (1985) listed several factors affecting the efficiency of N fertilization in increasing plant CP, including climatic factors (temperature, rainfall, light), soil characteristics (chemical and physical), plant enzyme activity, N concentration in the soil, metabolic activity of the plant, root geometry, and factors associated with N uptake and utilization by forage plants. Harkess and Frame (1986)

added to that list the frequency and severity of defoliation and type of grassland. Andrew and Johansen (1978), also included the availability of water as a major determinant of response to N of plants growing in the field.

One of the biggest concerns of the scientific community today is finding ways to reduce contamination of the environment by chemical products utilized on crops and grasslands. The inefficient utilization of applied N by ruminants grazing grasslands has been questioned, since only 14 to 17% of N inputs are normally recovered in animal products (Leaver and Weissbach, 1993). The remainder is either incorporated into soil OM or lost through leaching into watercourses, volatilization as ammonia, and volatilization as the products of denitrification (Scholefield et al., 1988). Ball and Ryden (1984) estimated that 300 kg N ha^{-1} yr^{-1} can be lost from intensively managed grazed grassland. On the other hand, Teitzel (1991) proposed that nitrate leaching under grazed tropical pastures will be minimal if N rates are less than 300 kg N ha^{-1} yr^{-1} and N is applied during the growing season when the grass demand for N is high. In agreement with this suggestion Scholefield et al. (1988) indicated that low N losses are feasible for N inputs in the range of 50 to 250 kg N ha^{-1} yr^{-1}.

In terms of economic limits for efficient N fertilizer use in grazing systems, Hodgson (1990) suggested rule of thumb amounts of 300 kg ha^{-1} yr^{-1} for milk production and 200 kg ha^{-1} yr^{-1} for beef and sheep production. He also stated that in efficient grazing systems, each extra kg of N used should result in an increased output of 10 L of milk or 1 kg of liveweight gain.

Pasture-based systems are undoubtedly the cheapest way to produce protein for ruminants and supplementation is not always economically feasible. Because of that, two alternatives proposed to overcome the limitation of CP in limpograss pastures are N fertilization and legume overseeding. Rotational grazing in combination with N fertilization after each defoliation was proposed by Sollenberger et al. (1989) as a possible management practice to improve animal performance on limpograss. Rusland et al. (1988) obtained 80% higher ADG for steers grazing limpograss-aeschynomene pastures than those grazing N-fertilized rotationally stocked limpograss. Even with this impressive result supporting a grass-legume association, several authors (Sollenberger et al., 1987b; Kalmbacher et al., 1988; Chaparro et al., 1991, 1992) have noted problems in long-term stand survival of aeschynomene and difficulties in initial establishment of the legume. Analyzing efficiency and economics of grass-legume associations in the tropics, Teitzel (1991) indicated that N-fertilized grass pastures attain higher animal production compared with tropical grass-legume pastures. Some of his results demonstrated that profits from grass-N systems were around \$200 ha^{-1} yr^{-1} higher than for grass-legume associations.

The unbalanced DOM/CP ratio of limpograss does not favor optimal rumen fermentation conditions. Cellulolytic microorganisms reduce their activity at low levels of ammonia release and this is a major problem for high fiber diets. Taking into account that limpograss NDF concentrations are generally over 700 g kg^{-1} of DM and also that a large proportion of limpograss N is associated with the fiber fraction (Holderbaum, 1989), it is not difficult to understand why grass utilization has not

been efficient. One of the hypotheses of the present research is that an increase in N fertilization rate of limpograss pastures will decrease the forage DOM/CP ratio through an increase in CP concentration and no effect or a slight decrease in DOM concentration. Considering that N fertilization requires less labor input than supplementation, one of the objectives of this research is to determine if N fertilization alone can assist in overcoming limpograss N deficiency and improve animal performance.

Nitrogen Supplementation of Cattle Grazing Warm-Season Grasses

Warm-season grasses are well adapted to low input beef cattle systems in the tropics and subtropics. Economically they are attractive due to their low investment and maintenance costs, and low labor requirements. Quantitatively they have high DM yield, excellent persistence, and good response to N fertilization. Qualitatively, they are not always capable of providing enough energy and protein to support desired performance of grazing ruminants. Nutritive value of WSG used as summer pasture or hay in Florida often is not adequate to meet requirements of different classes of cattle in the state (Moore, 1992). Ward and Klopfenstein (1991) suggested that nutritional management of beef cattle involves grazing to the extent possible and feeding supplements when necessary.

Supplements can be used to correct a specific nutrient deficiency in grazed herbage or to provide a smooth transition from one feeding regime to another (Hodgson, 1990). Besides the ability to enable animals to survive, to produce, and to

reproduce, Siebert and Hunter (1982) proposed that supplements can increase the consumption of available herbage or allow the animal to metabolize the same quantity of herbage more efficiently. Gill et al. (1989) suggested that the criteria for initiating supplementation include inadequacy of overall supply of metabolizable energy (ME) from forages or when changes in the composition of the final product are desired. The necessity to nourish rumen microorganisms and to obtain an adequate supply of digestible essential amino acids in the gut also were proposed by Church (1991) as reasons to use protein supplementation. Possible modes of supplement action were listed by Egan (1976) and include increasing energy intake directly through the digestible energy (DE) concentration of supplement, increasing digestibility of the basal diet, increasing voluntary intake of the basal diet, and improving the efficiency of energy use by changing proportions of nutrients. Additionally, supplements can increase pasture carrying capacity, provide a carrier for growth-promoting additives, and aid in prevention or treatment of health problems (Lusby, 1990; cited by Patterson et al., 1994).

Formulation of supplements for grazing ruminants can be difficult. Ellis (1990) listed the following items as required information before a supplement can be formulated: animal and ruminal microbial requirements, degradable and undegradable protein in the forage, available energy intake from forage, and interactions between the forage and supplement. The laborious nature and doubtful accuracy of the methods used to measure herbage intake of grazing animals were indicated by Meijs (1981) as the major reasons for the paucity of available information for pasture-based

systems. Without good estimates of intake, formulation of appropriate supplement rations is difficult. As noted by Ørskov (1976), one of the principal measurements required to define the type and amount of supplementation needed is the contribution of the basal diet (microbial protein and UDP) to the requirement of the host.

According to Bates et al. (1988) the response to a protein supplement of ruminant livestock fed low quality tropical grass varies substantially and depends on the rumen degradability of the protein source and DOM intake. It has been well demonstrated that manipulation of the rumen environment via different diet sources is a viable alternative to increase intake, improve feed efficiency, and consequently, obtain better animal performance. Crude protein and non-structural carbohydrates are examples of dietary components that can be manipulated to optimize ruminal fermentation and to increase the passage of amino acids to the small intestines (Clark et al., 1992).

When a supplement is provided, forage intake may either increase, remain unchanged, or decrease, and many factors affect the direction and extent of the change (Moore, 1992). In the opinion of Hodgson (1990), there are very few circumstances in which the utilization of conventional concentrates or forages as supplements do not decrease herbage intake (substitution effect). Proportion of concentrate, type of roughage, and type of concentrate are the three factors described by Faverdin et al. (1991) which influence substitution rates. Owens et al. (1991) found less depression in forage intake with protein supplements because they cause less depression in ruminal pH, fiber digestion rate, and ruminal ammonia

concentration. The DOM/CP ratio of the forage was identified by Moore and Kunkle (1995) as a major factor affecting the change in intake due to feeding supplements. They proposed three different scenarios for which supplementation will result in variable responses. If DOM/CP ratio is high (>7) small amounts of protein supplement may increase forage intake. Secondly, larger amounts of concentrate, particularly those containing starch, may decrease forage intake. Finally, for forages with a balanced DOM/CP ratio (<7) forage intake will decrease in proportion to the amount of concentrate fed. Taking these scenarios in consideration, the high DOM/CP ratio of limpograss herbage provides an excellent opportunity for improving intake and animal performance with limited CP supplementation.

Reduction in intake of animals grazing forages deficient in CP seems to be caused by low availability of amino acids at the tissue level rather than by digestibility per se (Minson, 1982; Nolan and Leng, 1989). Owens et al. (1991) pointed out that central nervous system signals may be involved in intake control of low quality forages, and that fermentation in the intestinal tract and N recycling might be involved. Studies with sheep by Miner et al. (1990), quoted by Owens (1991), and Hannah et al. (1990) with cattle reported that no more than 60% of ruminal capacity of grazing ruminants was occupied. Their data do not support the theory of ruminal fill limiting forage intake in grazing animals. Kunkle (1993b) summarized several studies in Florida that showed a 15 to 45% increase in forage intake due to protein supplementation.

The interaction of available protein and energy is a complex process that has a major influence on herbage intake and animal performance. Preston (1976) considered the rate of entry into the rumen of fermentable carbohydrates as the principal constraint limiting production of microbial protein. Hogan and Weston (1981) stated that N limitation to microbial protein synthesis appears likely when the DOM/CP ratio in the diet exceeds 10. For gains in beef cattle of 0.5, 1.0, and 1.5 kg d^{-1}, 150-kg calves need a CP/TDN ratio of, respectively, 0.20, 0.23, and 0.25 (Owens et al., 1991). Galyean and Goetsh (1993) proposed that low fermentability of WSG normally limits volatile fatty acid (VFA) production and general animal energy status.

After a comprehensive review of the international literature, Klopfenstein (1993) proposed a general requirement for ruminal degradable protein of 13% of the diet TDN. Using a different approach, Kunkle et al. (1994) reported that these requirements are usually met when the diet TDN/CP ratio is seven or lower and concentration of PUN is 10 mg dL^{-1} or higher. Controversial information has been published on how efficiently NPN (especially urea) can be used to supply these requirements. After extensive review on this topic, Holderbaum (1989) concluded that the effectiveness of urea as a dietary N provider will depend on the source of readily fermentable carbohydrate and ratio of total dietary DOM to CP. As a supplement to low quality forages, NPN is considered an inferior source compared with intact protein (Owens et al., 1991; Church, 1991; Kunkle, 1993b). The efficiency of NPN utilization by ruminants was evaluated by Satter and Roffler

(1977). They concluded that NPN may be utilized as well as true protein when ruminal NH_3-N concentration is low (<5 mg dL^{-1}). When levels are in excess of this value, supplementation with NPN was without benefit. From a practical standpoint, these authors recommended the addition of NPN to high concentrate rations containing less than 12 to 13% CP or to all forage rations containing less than 9 to 10% CP. Comparing responses of calves grazing bahiagrass during summer in Florida and fed either molasses-urea or molasses-feather meal-urea supplements, Pate (1991) did not find any advantage for the natural protein over NPN. Ruminal degradability of N was presented by Bates et al. (1988) as an important determinant of efficiency of molasses-urea supplementation of sheep receiving low quality limpograss hay. In a grazing situation, Holderbaum (1989) compared performance of steers fed no supplement on limpograss pastures with those supplemented with corn (0.587 kg d^{-1}) and two levels of urea (123 or 500 g CP kg^{-1} supplement). He reported no difference in steer weight gain between levels of urea but a 100% increase in seasonal daily gains for supplemented over the unsupplemented treatment. However, corn-urea supplementation did not eliminate the summer slump in performance during the period when ADG was lowest for unsupplemented steers.

Several studies have demonstrated that escape or UIP may be utilized more efficiently than more readily degraded protein sources to meet requirements of growing animals (Kaufman and Lupping, 1982; Linda and Paterson, 1986; Hardin et al., 1989; Nolan and Leng, 1989; Goetsch et al., 1990; Rock et al., 1991). Additional gain obtained by steers grazing summer range and fed escape protein

indicated that microbial protein synthesis may be unable to satisfy the metabolic protein requirement of the animals (Karges et al., 1992). The success or failure of feeding protected proteins will depend on the energy and N fractions in feeds (Clark et al., 1992). An estimate of N supplement needed can be obtained by measuring NH_3 concentration in the rumen fluid. Several ranges of NH_3 concentration have been proposed to optimize microbial growth and organic matter digestion. These are 5 to 8.5 mg NH_3 dL^{-1} (Satter and Slyter, 1974; Roefler and Satter, 1975), 5 to 7 (Ørskov, 1976), and 3.3 to 8.0 (Hoover, 1980). Low NH_3 availability can reduce structural carbohydrate digestion and ultimately intake because cellulolytic microorganisms are highly dependent on NH_3-N (Nocek and Russell, 1988). Klopfenstein et al. (1991) mentioned that advantages of using escape protein include a reduction in the amount of natural protein needed, an increase in utilization of lower cost urea, lower cost of supplementation, and maintenance of the same performance. Studying some high escape proteins as sources of N and amino acids, Titgemeyer et al. (1989) concluded that differences among protein sources will be of great importance as nutritionists attempt to define and meet the amino acid needs of ruminants.

It is important to remember that escape protein supplements have limited value if N requirements of the rumen microflora have not been met (Beever, 1989). Despite undergoing rapid and extensive ruminal degradation, Brown and Pitman (1991) reported low absolute quantities of ruminally soluble and degradable N for bahiagrass and limpograss, which may limit microbial protein synthesis in the rumen.

Schingoethe (1991) listed three basic points to be remembered about escape protein supplementation. First, it is important that protein not bypass the rumen at the expense of rumen microbial production. Second, some escape protein may be undigestible and not usable in the lower digestive tract. Third, the escape protein should have at least comparable or superior quality (pool of amino acids) than the rumen microbial protein. As a feeding strategy for beef production systems, Petit and Flipot (1992) and Kunkle et al. (1994) proposed a maximization of microbial protein synthesis via NPN and a provision of UIP when required by the animals and if it is economically feasible. McCollum and Horn (1990), quoted by Owens (1991), considered possible estimations of microbial protein synthesis and flow of protein to the intestines for different forages. However, they stated that at present, practical information is limited to aid in formulation of supplements to meet needs of ruminants. Actually, very little information is available relating degradable and undegradable protein supplements with performance of cattle grazing WSG. To aid in acquiring more information a second hypothesis to be tested in the present research is that adding undegradable protein (corn gluten meal and blood meal) to a corn-urea supplement will affect forage intake and ADG of heifers grazing limpograss in the summer.

CHAPTER 3
INFLUENCE OF NITROGEN FERTILIZATION AND SUPPLEMENTATION ON LIMPOGRASS FORAGE QUALITY AND UTILIZATION BY BEEF REPLACEMENT HEIFERS

Introduction

Grazed herbage is the primary source of nutrients for ruminants in warm climates throughout the world. Constraints associated with pasture-based systems normally include seasonality of forage production and low quality of the available herbage. Reviewing tropical grass literature, Norton (1982) and Minson (1990) concluded that only half of the tropical grass samples described would meet the crude protein (CP) concentration recommended for cattle maintenance. Concentrations of N below 10 g kg^{-1} dry matter (DM) affect the fermentative efficiency of rumen bacteria and reduce herbage intake and digestion (Hodgson, 1990). Tropical perennial grasses used as summer pasture or hay in Florida were described by Moore (1992) as having insufficient nutrient composition and quality to meet the nutrient needs of grazing ruminants. Strategic supplementation of cattle grazing tropical grass pastures is one means of addressing these needs.

'Floralta' limpograss [*Hemarthria altissima* (Poir.) Stapf. et C. E. Hubb.] is growing on an estimated 50,000 ha in Florida (Quesenberry, 1993). It was introduced into Florida beef production systems due to positive attributes including

vigorous growth, high DM production, relatively high digestibility, good adaptation to poorly drained soils, and a longer grazing period than most other grasses in fall. It has been well documented, however, that low herbage CP concentrations of Floralta limpograss limit intake and gains of growing animals grazing pastures during summer (Rusland et al., 1988; Sollenberger et al., 1988, 1989; and Holderbaum et al., 1991). Several management alternatives have been tested in order to address the CP limitation and associated high digestible organic matter (DOM)/CP ratio of Floralta limpograss. Use of the legume aeschynomene (*Aeschynomene americana* L.) in association with limpograss (Rusland et al., 1988) and feeding N supplement to livestock grazing limpograss pastures (Holderbaum et al., 1991) have improved cattle productivity, but the slump in animal performance during summer was not totally eliminated.

Because low herbage N concentration and high DOM/CP ratio have been associated with poor performance of cattle grazing limpograss, increasing pasture N fertilization rates may be an alternative management practice to feeding N supplements. No data are available that document the effect of pasture N fertilization rate on daily gains of cattle grazing limpograss. Additionally, supplementation efforts with cattle grazing limpograss have focused on rumen degradable N sources. Research is needed to determine if N supplements including rumen undegradable protein will result in further increases in animal performance. The objectives of this study were to test the following hypotheses: i) Increasing N fertilization rate of limpograss pastures increases herbage CP concentration, has no effect on in vitro

digestible organic matter (IVDOM) concentration, decreases herbage DOM/CP ratio, and increases the average daily gain (ADG) and plasma urea N concentration (PUN) of heifers grazing 4-wk limpograss regrowth and receiving no N supplement; ii) Average daily gain of yearling beef heifers grazing limpograss is improved by adding rumen undegradable protein to a mainly rumen degradable protein supplement of corn plus urea.

Material and Methods

The experiment was carried out during July to October of 1992 and 1993 at the University of Florida's Beef Research Unit located northeast of Gainesville, Florida (29° 60'N lat). Pastures used in this study were existing Floralta limpograss swards established between 1980 and 1986. Soils at the research site are moderately to poorly drained flatwood types. They include sandy Spodosols of low fertility, primarily belonging to the Pomona and Smyrna series. These soils have an underlying impermeable layer about 30 to 60 cm below the surface, and during the summer rainy season standing water is quite common. Prior to initiating the experiment in 1992, soil pH was 5.8, and Mehlich I extractable P and K levels were 18 and 26 mg kg^{-1}, respectively. In 1993, soil pH was 5.3, and Mehlich I extractable P and K levels were 19 and 12 mg kg^{-1}. Fertilizer was applied in April of each year. Forty-five kg N ha^{-1} and 75 kg K ha^{-1} were applied in April 1992 and the same amounts of N and K plus 20 kg P ha^{-1} were applied in April of 1993. The total experimental area encompassed 4.8 ha with twelve experimental units measuring 0.4

ha each. The 0.4-ha pastures were divided with electric fence into five paddocks of equal size (0.08 ha). A 35-d rotational grazing cycle was used with 7-d of grazing on each paddock followed by a 28-d rest period. The pastures were staged during a preliminary period of one grazing cycle and the experiment was from 7 July to 1 Oct. 1992 (86 d). In 1993 after a preliminary period of 28-d (from 21 May to 17 June), animals were removed from the pastures for 2 wk due to low rainfall (Table 3.1) and insufficient herbage mass to support the testers. The experiment began on 1 July and continued for 97 d to 6 Oct. 1993.

The experimental variables tested were two rates of N fertilization (50 and 150 kg ha^{-1} during the summer) and three supplements (NONE, corn + urea [CU], and corn + urea + rumen undegradable protein sources [CUUP]), combined as a 2 X 3 factorial in a completely randomized design with two replications. Throughout the text the six treatment combinations will be referred as 50NONE, 150NONE, 50CU, 150CU, 50CUUP, and 150CUUP. Sources of undegradable protein used were corn gluten meal and blood meal. Nitrogen was applied as ammonium nitrate (NH_4NO_3) in three equal applications (before the first, second, and third grazing cycles) to achieve totals of 50 and 150 kg N ha^{-1} during the summer of each year (45 kg N ha^{-1} in April is not included in this total).

The two protein supplements (CU and CUUP) were calculated based on total digestible nutrient (TDN) and CP requirements of a 350-kg heifer gaining 0.45 kg d^{-1} (i.e., 6.8 kg d^{-1} of dry matter intake [DMI], 4.13 kg d^{-1} of TDN, and 0.576 kg d^{-1} of CP) (Kunkle et al., 1993). Ground corn (95 g CP kg^{-1} and 870 g TDN kg^{-1}), corn

gluten meal (700 g CP kg^{-1} and 870 g TDN kg^{-1}), blood meal (850 g CP kg^{-1} and 680 g TDN kg^{-1}), feed grade urea (2870 g CP kg^{-1}), sulphur (dynamate), Ca and P, were used in formulating the protein supplements. The supplements were formulated to be isocaloric and to provide approximately the same rumen degradable protein (0.27 kg d^{-1}) and 0.05 (CU) and 0.14 kg d^{-1} (CUUP) of rumen undegradable protein (calculated values). In that way if a higher response in animal performance was obtained on the CUUP supplement compared to CU, it would be indicative of a response to additional undegradable intake protein (UIP). Supplements were fed at 0.7 kg DM per head d^{-1} and had CP concentrations of 409 (CU) and 575 g kg^{-1} (CUUP). Tables 3.2, 3.3, and 3.4 describe the composition and ingredients for each supplement.

Table 3.1. Monthly rainfall totals (March to October) in 1992 and 1993 at the Beef Research Unit, Gainesville, Florida.

Month	Year		
	1992	1993	70-yr average
	mm		
March	156	102	93
April	95	49	74
May	64	54	106
June	303	100	167
July	69	106	176
August	193	140	204
September	126	55	143
October	169	116	59
Total	1175	722	1022

Table 3.2. Composition of supplements on a dry matter basis.

Supplement	Corn	Urea	CGM†	BM‡	S	$CaCO_3$
	----------	----------	----------	g kg^{-1} ----------	----------	----------
CU	849	125	-	-	22	4
CUUP	429	92	355	95	23	6

† Corn gluten meal
‡ Blood meal

Table 3.3. Daily supplement and nutrients fed.

Supplement	As fed	DM†	CP‡	DIP§	UIP¶	TDN#
	----------	----------	kg $head^{-1}$ d^{-1}	----------	----------	----------
CU	0.79	0.70	0.31	0.27	0.04	0.52
CUUP	0.77	0.70	0.44	0.27	0.17	0.52

† Dry matter
‡ Crude protein
§ Degradable intake protein
¶ Undegradable intake protein
# Total digestible nutrients

Table 3.4. Proportion of total supplement crude protein (CP) contributed by each supplement ingredient.

	Supplement	
Ingredient	CU†	CUUP‡
	---- g ingredient CP kg^{-1} supplement CP ----	
Corn	184	64
Urea	816	418
CGM§	-	391
BM¶	-	127

† Corn + urea
‡ Corn + urea + undegradable protein
§ Corn gluten meal
¶ Blood meal

A variable (put and take) stocking rate was used to achieve a pasture stubble height of approximately 20 to 25 cm on each paddock at the end of a grazing period. Grazing frequency and stubble height were selected based on previous experiments (Rusland et al., 1988; Sollenberger et al., 1989; and Holderbaum et al., 1991). Two crossbred 13- to 16-mo-old, pregnant (60 to 90 d) beef heifers were assigned to each pasture. Heifers were 25 to 50% Brahman (*Bos indicus)* with Angus and Hereford as the dominant *Bos taurus* breeds. Average liveweight of testers was 350 kg at the start of grazing and testers remained on the pastures during the entire grazing season. Additional grazers (put and takes) were added as needed (Mott and Lucas, 1952) to achieve the desired stubble height. All the pastures contained a shade structure, waterer, and feeder containing a salt-based trace mineral mixture. Minerals were supplied free choice throughout the whole grazing season.

Shrunk weights (16-h fast on dry lots) of all animals were taken at the start of the trial and at 28-d intervals during the experimental period each year. On weigh dates, blood samples were collected (10 mL) from tester animals by jugular venipuncture. The plasma was separated by centrifugation, frozen, and stored at -20°C for PUN analysis. Samples were analyzed by an automated colorimetric procedure (Technicon Autoanalyzer II Industrial Method n° 339-01, Technicon Instruments Corp., Tarrytown, NY) based on the diacetyl monoxime method of Marsh et al. (1965). Put and take animals also were weighed when added to or removed from pastures.

All ADG calculations were made using performance data of tester animals only. Total beef production per hectare (GAIN) was calculated by multiplying ADG of tester heifers by animal grazing days per hectare (both testers and put and takes). Animal grazing days per hectare (carrying capacity) was determined by calculating the 100-kg liveweight days per hectare of both tester and grazer animals and dividing by four. Thus carrying capacity is expressed on the basis of number of 400-kg animals.

Throughout both grazing seasons, pregraze and postgraze herbage mass samples were collected on the second and fifth paddocks (i.e., two times per grazing cycle). In each paddock sampled, eight 0.25-m^2 quadrats were selected that represented mean herbage mass and pasture condition. Herbage in each of the eight quadrats was clipped to a 15-cm stubble height. Herbage from the two samples per paddock were dried at 60°C to constant weight. Herbage growth (kg ha^{-1} d^{-1}) was calculated as change in herbage mass during the preceding rest period (pregraze herbage mass of the current grazing cycle minus postgraze herbage mass of the previous grazing cycle) divided by days in the rest period (28). Herbage allowance was calculated as average herbage mass (kg ha^{-1} d^{-1}) above a 15-cm stubble (0.5 times the sum of pregraze herbage mass of the current grazing cycle plus postgraze herbage mass of the current grazing cycle) divided by average number of kg of animal liveweight during that period (kg ha^{-1} d^{-1}). Herbage consumed during a given grazing cycle was calculated as the sum of herbage mass that disappeared during the grazing period (pregraze herbage mass minus postgraze herbage mass) plus herbage growth

during that period (kg ha^{-1} d^{-1}*7). Estimated intake was calculated as herbage consumed (kg ha^{-1} d^{-1}) divided by the average number of kg of animal liveweight (kg ha^{-1} d^{-1}) during that period. Herbage allowance and estimated intake are expressed as a percentage of animal liveweight and are averages across a grazing season (1992 and 1993). Herbage consumed is the sum across a grazing season.

Pregraze handplucked samples were taken at 20 sites at each sampling of the second and fifth paddocks. Herbage was harvested at the target postgraze stubble height (20-25 cm), dried in a forced-air oven at 60°C to constant weight, and ground to pass a 1-mm screen. A second handplucked sample from Paddock 5 was taken in each grazing cycle, and separated into limpograss leaf blade and limpograss stem plus sheath fractions. Crude protein and IVDOM, and neutral detergent fiber (NDF) were determined on the handplucked samples from Paddocks 2 and 5 and for the fractions (leaf blade and stem plus sheath) from Paddock 5. In vitro digestible organic matter was determined using a modified two-stage digestion procedure (Moore and Mott, 1974). A modified aluminum block digestion procedure (Gallaher et al., 1975) and semi-automated colorimetry (Hambleton, 1977) were used for all N determinations. Crude protein was calculated as N times 6.25 and is expressed on a DM basis. Determination of NDF followed the procedure described by Golding et al. (1985).

Data were analyzed by using analysis of variance in PROC GLM (General Linear Models Procedure) of the Statistical Analysis System (SAS; SAS Institute Inc., 1989). Year was included in the model as a subplot treatment in a split-plot arrangement of the completely randomized design. Nitrogen by supplement treatment

combination was the main plot. For ADG, GAIN, carrying capacity (CC), and PUN comparisons were made using the planned single degree of freedom contrasts 50 vs. 150, 50NONE vs. 150NONE, CU vs. CUUP, 50CU + 50CUUP vs. 50NONE, and 150CU + 150CUUP vs. 150NONE (considered different when $P \leq 0.10$). Herbage nutritive value means from handplucked samples were compared using the same contrasts (considered different when $P \leq 0.05$). When planned comparisons of 50 vs. 150 and CU vs. CUUP were not valid due to N rate x supplement interactions, the PDIFF option was used for making comparisons of interest within one level of a given factor.

Results

Herbage Nutritive Value (1992-1993)

A minimal year effect was observed for herbage CP ($P = 0.0275$) (Table 3.5), with concentrations in 1993 being slightly greater (66 g kg^{-1}) than in 1992 (63 g kg^{-1}). There also was an N rate by supplement interaction for herbage CP ($P = 0.0277$).

This interaction occurred because the magnitude of the increase in CP with the higher N fertilizer rate was not consistent across supplement treatments, ranging from 10 to 25 g CP kg^{-1} (Table 3.6). There was a trend for herbage from supplemented treatments to have greater CP ($P=0.1117$; and $P=0.1450$) (Table 3.6).

Herbage IVDOM was affected by N rate and year (Table 3.5). Pastures fertilized at 150 kg N ha^{-1} had higher herbage IVDOM than those fertilized at 50 kg N ha^{-1} (Table 3.7). Herbage in 1993 had higher IVDOM (539 g kg^{-1}) than in 1992 (511 g kg^{-1}).

Table 3.5. Levels of probability (P value) for effects of N fertilization (N), supplementation (SUP), year (YEAR) and their interactions on handplucked herbage crude protein (CP), herbage in-vitro digestible organic matter (IVDOM), herbage neutral detergent fiber concentration (NDF), herbage digestible organic matter:crude protein ratio (DOM/CP), and herbage leaf (blade)/stem + sheath ratio (LEAF/STEM) .

	Response variable				
Effect	CP	IVDOM	DOM/CP	NDF	LEAF/STEM
N	0.0001	0.0020	0.0001	0.0065	0.0084
SUP	0.0802	0.0955	0.0147	0.6072	0.1735
N*SUP	0.0277	0.6150	0.0140	0.1508	0.1581
YEAR	0.0275	0.0001	0.9873	0.1402	0.0007
N*YEAR	0.2087	0.2027	0.4349	0.8817	0.7942
SUP*YEAR	0.7989	0.4593	0.9239	0.3019	0.3764
N*SUP*YEAR	0.3774	0.1023	0.6920	0.4327	0.1592

Table 3.6. Interaction of pasture N fertilizer rate and supplement treatment on herbage crude protein. Data are means across the 1992 and 1993 grazing seasons (n=4).

	Supplement[†]		
N rate	NONE	CU	CUUP
kg ha^{-1}	----------	g kg^{-1}	----------
50	53[‡]	60	55
150	70	70	80

Results of significance tests (P values) of planned comparisons among treatment means:

50NONE vs. 150NONE	0.0008
50 vs. 150	0.0001[§]
CU vs. CUUP	0.2459[§]
50CU + 50CUUP vs. 50NONE	0.1450
150CU + 150CUUP vs. 150NONE	0.1117

[†] NONE, CU, and CUUP are no supplement, corn + urea, and corn + urea + rumen undegradable protein, respectively.
[‡] Standard error of an interaction mean equals 1.9.
[§] Main effect comparisons that were not used because of interaction.

Table 3.7. Main effects of pasture N fertilizer rate and supplement treatment on herbage in-vitro digestible organic matter concentration of limpograss handplucked herbage. Data are means across the 1992 and 1993 grazing seasons.

	Supplement†			
N rate	NONE	CU	CUUP	Mean‡
kg ha^{-1}	----------	g kg^{-1} OM		----------
50	516	499	511	509
150	544	528	554	542
Mean§	530	514	533	

Results of significance tests (P values) of planned comparisons among treatment means:

50NONE vs. 150NONE	0.0422
50 vs. 150	0.0020
CU vs. CUUP	0.0049
50CU + 50CUUP vs. 50NONE	0.3079
150CU + 150CUUP vs. 150NONE	0.7364

† NONE, CU, and CUUP are no supplement, corn + urea, and corn + urea + rumen undegradable protein, respectively.

‡ Standard error of a N rate treatment mean (across supplements and years) equals 4.54 (n=12).

§ Standard error of a supplement treatment mean (across N rates and years) equals 5.57 (n=8).

The DOM/CP ratio of handplucked herbage was affected by an N rate by supplement interaction (Table 3.5). The major factor influencing the response was N rate, and DOM/CP was greater for the lower N fertilization treatment across supplements (Table 3.8). Within an N rate, DOM/CP was or tended to be lower for CU and CUUP treatments than for NONE. This was related to the trend toward higher herbage CP concentration on the pastures where heifers were supplemented.

Neutral detergent fiber (NDF) was affected only by N fertilizer rate ($P=0.0065$, Table 3.5). Pastures fertilized with 150 kg N ha^{-1} had a lower NDF concentration (790 g kg^{-1}) compared with those fertilized at 50 kg N ha^{-1} (805 g kg^{-1}). The overall mean NDF concentration of 797 g kg^{-1} across years and treatments provides perspective on the magnitude of the cell wall fraction in limpograss herbage DM.

The utilization of the higher N fertilizer rate (150 kg N ha^{-1}) compared with the lower (50 kg ha^{-1}) increased leaf/stem ratio from 0.31 to 0.39 ($P= 0.0084$) (Table 3.9). The increase in leaf percentage promoted by the higher N fertilizer rate is important because leaf blade had mean CP across years of 106 g kg^{-1}, while mean CP of stem + sheath was only 45 g kg^{-1} (Table 3.10). The increase in leaf/stem ratio when 150 kg N ha^{-1} was applied also was responsible for much of the decrease in DOM/CP ratio associated with the higher N rate. Crude protein of leaf blades was more than two-fold greater than that of stem plus sheath while IVDOM of the two fractions was nearly the same (511 g kg^{-1} for stem plus sheath and 508 g kg^{-1} for leaf blade) (Table 3.10). Nitrogen and year were the most important factors affecting

nutritive value responses of pasture fractions (leaf blade and stem + sheath) (Table 3.11). Higher N rate increased leaf and stem + sheath CP, had no effect on leaf IVDOM and NDF, increased stem + sheath IVDOM, and decreased stem + sheath NDF (Table 3.10).

Table 3.8. Interaction of pasture N fertilizer rate and supplement treatment on digestible organic matter (DOM)/crude protein (CP) ratio of limpograss handplucked herbage. Data are means across the 1992 and 1993 grazing seasons (n=4).

	Supplement†		
N rate	NONE	CU	CUUP
kg ha^{-1}	----------	DOM/CP	----------
50	9.7‡	8.4	9.3
150	7.7	7.6	7.0

Results of significance tests (P values) of planned comparisons among treatment means:

50NONE vs. 150NONE	0.0003
50 vs. 150	0.0001§
CU vs. CUUP	0.3308§
50CU + 50CUUP vs. 50NONE	0.0092
150CU + 150CUUP vs. 150NONE	0.0792

† NONE, CU, and CUUP are no supplement, corn + urea, and corn + urea + rumen undegradable protein, respectively.
‡ Standard error of an interaction mean equals 0.18.
§ Main effect comparison that was not used because of interaction.

Table 3.9. Main effects of pasture N fertilizer rate and supplement treatment on leaf blade/stem + sheath ratio of limpograss handplucked herbage. Data are means across the 1992 and 1993 grazing seasons.

	Supplement[†]			
N rate	NONE	CU	CUUP	Mean[‡]
kg ha^{-1}	----------	ratio	----------	
50	0.30	0.34	0.30	0.31
150	0.34	0.38	0.44	0.39
Mean[§]	0.32	0.36	0.37	

Results of significance tests (P values) of planned comparisons among treatment means:

50NONE vs. 150NONE	0.2628
50 vs. 150	0.0084
CU vs. CUUP	0.5924
50CU + 50CUUP vs. 50NONE	0.5523
150CU + 150CUUP vs. 150NONE	0.0571

[†] NONE, CU, and CUUP are no supplement, corn + urea, and corn + urea + rumen undegraded protein, respectively.

[‡] Standard error of a nitrogen rate treatment mean (across supplements and years) equals 0.022.

[§] Standard error of a supplement treatment mean (across N rates and years) equals 0.027.

Table. 3.10. Main effect of N fertilizer rate on crude protein, in-vitro digestible organic matter, and neutral detergent fiber of limpograss herbage fractions (leaf blade and stem + sheath). Data are means across supplements and years.

	Leaf			Stem + sheath		
	CP	IVDOM	NDF	CP	IVDOM	NDF
N rate	----------		g kg^{-1}	----------		
50	97	495	753	38	489	835
150	115	521	749	51	533	815
P value	0.0004	0.0827	0.5736	0.0022	0.0076	0.0143

Table 3.11. Levels of probability (P value) for effects of N fertilization (N), supplementation (SUP), year (YEAR) and their interactions on handplucked herbage leaf blade crude protein (CPL), stem + sheath CP (CPS), herbage leaf blade in-vitro digestible organic matter concentration (IVDOML), herbage stem + sheath IVDOM (IVDOMS), herbage leaf blade neutral detergent fiber (NDFL), and herbage stem + sheath NDF (NDFS).

	Response Variable					
Effect	CPL	CPS	IVDOML	IVDOMS	NDFL	NDFS
N	0.0004	0.0022	0.0827	0.0076	0.5736	0.0143
SUP	0.1790	0.1180	0.3659	0.1525	0.3374	0.2728
N*SUP	0.5616	0.3581	0.6325	0.6090	0.5658	0.2870
YEAR	0.0066	0.0004	0.0152	0.0001	0.0046	0.0001
N*YEAR	0.1351	0.0377	0.8497	0.2261	0.3993	0.0736
SUP*YEAR	0.3618	0.0682	0.5534	0.5728	0.2675	0.1275
N*SUP*YEAR	0.9094	0.2649	0.8652	0.1355	0.5566	0.0152

Animal Performance and Herbage Mass Responses (1992-1993)

There was an N fertilization rate by supplement interaction (P=0.0207, Table 3.12) for ADG across the 1992 and 1993 grazing seasons. Interaction occurred because gains of supplemented heifers were not different from those of unsupplemented heifers (P=0.2556) when pastures received 150 kg N ha^{-1}, but ADG of unsupplemented heifers was less than supplemented heifers (P=0.0005) when pasture N rate was 50 kg ha^{-1} (Table 3.13). Heifers receiving the CUUP supplement had higher ADG than those receiving CU if fertilization rate was 50 kg N ha^{-1} (P=0.0813), but there was no difference when the rate was 150 kg N ha^{-1} (P=0.2954).

Using the sward difference technique to estimate intake did not provide sufficient precision to detect effects of treatments or year (data not shown). The mean estimated intake across treatments and years expressed as a percentage of heifer liveweight was 1.62%. This intake is lower than expected based on observed ADG and requirements of the animals for DMI, TDN, and CP from forage.

A variable stocking rate was employed in this experiment so that comparisons among treatments could be made at equal levels of grazing pressure or herbage allowance (HA). Results indicate no significant effects of N, supplement (SUP), or N by SUP interaction on HA response (Table 3.12). There was a year effect (P=0.0006) with a lower HA in 1992 (3.0% of animal liveweight) than in 1993 (3.7%).

Table 3.12. Levels of probability (P value) for effects of N fertilization (N), supplementation (SUP), year (YEAR), and their interactions on heifer average daily gain (ADG), intake (INT), herbage allowance (HA), plasma urea N (PUN), carrying capacity (CC), herbage consumed (HC), and gain per hectare (GAIN).

	Response variable						
Effect	ADG	INT	HA	PUN	CC	HC	GAIN
N	0.1656	0.5810	0.2542	0.0217	0.0518	0.0587	0.0084
SUP	0.0026	0.4742	0.3578	0.0001	0.5372	0.5610	0.0003
N*SUP	0.0207	0.5998	0.8247	0.1727	0.4938	0.2801	0.0067
YEAR	0.2378	0.8756	0.0006	0.0387	0.0375	0.6333	0.1171
N*YEAR	0.8202	0.5054	0.8937	0.8778	0.1480	0.2280	0.9274
SUP*YEAR	0.9211	0.6655	0.7024	0.1708	0.5859	0.6154	0.8957
N*SUP*YEAR	0.7745	0.3390	0.9227	0.3062	0.3388	0.3399	0.5880

Table 3.13. Interaction of pasture N fertilizer rate and supplement treatment on average daily gain of yearling heifers grazing limpograss. Data are means across the 1992 and 1993 grazing seasons (n=4).

	Supplement[†]		
N rate	NONE	CU	CUUP
kg ha^{-1}	---------	kg	---------
50	0.06[‡]	0.41	0.56
150	0.36	0.39	0.47

Results of significance tests (P value) of planned comparisons among treatment means:

50NONE vs. 150NONE	0.0009
50 vs. 150	0.1656[§]
CU vs. CUUP	0.0619[¶]
50CU + 50CUUP vs. 50NONE	0.0005
150CU + 150CUUP vs. 150NONE	0.2556

[†] NONE, CU, and CUUP are no supplement, corn + urea, and corn + urea + rumen undegradable protein, respectively.
[‡] Standard error of an interaction mean equals 0.050.
[§] Main effect comparison that was not used because of interaction.
[¶] Due to interaction, PDIFF was used to compare:
50CU vs. 50CUUP (P= 0.0813)
150CU vs. 150CUUP (P= 0.2954)
150NONE vs. 150CUUP (P= 0.0991)

Two-year means for heifer PUN concentration ranged from 4.2 (50NONE) to 17.4 mg (100 mL)$^{-1}$ (150CUUP), and PUN was affected by N rate, SUP, and YEAR (Table 3.12). Unsupplemented heifers grazing limpograss pastures fertilized with 50 and 150 kg N ha^{-1} had PUN concentrations of 4.2 and 9.2 mg (100 mL)$^{-1}$, respectively. For all supplemented treatments, heifer PUN concentrations were at least 15 mg (100 mL)$^{-1}$ (Table 3.14). No differences in PUN concentrations were observed among heifers receiving CU or CUUP supplements (P=0.3564), but supplemented heifers had higher PUN concentrations than unsupplemented heifers on both the 50NONE and 150NONE treatments.

There was only an N effect (P=0.0518) and a year effect (P=0.0375) for CC and no interactions. The greatest response of CC to N fertilizer occurred in 1992 where heifer days per hectare (based on a 400-kg heifer) were approximately 100 d greater for the higher (657 d ha^{-1}) than the lower N rate (558 d ha^{-1}) (Table 3.15). The weaker response to N fertilizer rate in 1993 can be explained by slower regrowth due to drought-stressed pastures during the grazing season (Table 3.1). Rainfall during June through September 1993 was 284 mm below the 70-yr mean, while during the same months in 1992 rainfall was 1 mm above the 70-yr mean. Despite less rainfall, CC was greater in 1992 than 1992, primarily due to a longer grazing season (97 vs. 86 d).

Table 3.14. Main effects of pasture N fertilizer rate and supplement treatment on plasma urea N concentration of yearling heifers grazing limpograss.

	Supplement[†]			
N rate	NONE	CU	CUUP	Mean[‡]
kg ha^{-1}	---------- mg (100 ml)$^{-1}$ ----------			
50	4.2	15.0	16.9	12.0
150	9.2	17.2	17.4	14.6
Mean[§]	6.7	16.1	17.2	

Results of significance tests (P values) of planned comparisons among treatment means:

50NONE vs. 150NONE	0.0137
50 vs. 150	0.0217
CU vs. CUUP	0.3564
50CU + 50CUUP vs. 50NONE	0.0001
150CU + 150CUUP vs. 150NONE	0.0007

[†] NONE, CU, and CUUP are no supplement, corn + urea, and corn + urea + rumen undegradable protein, respectively.

[‡] Standard error of a nitrogen rate main effect mean (across supplements and years; n=12) equals 0.59.

[§] Standard error of a supplement main effect mean (across N rates and years; n=8) equals 0.73.

Table 3.15. Main effects of pasture nitrogen fertilizer rate and year on carrying capacity of yearling heifers grazing limpograss. Data are means across supplements within the 1992 and 1993 grazing seasons (n=12).

	Year		
N rate	1992	1993	Mean[†]
kg ha^{-1} yr^{-1}	---------- heifer days ha^{-1} ----------		
50	558	639	599
150	657	676	667
Mean[‡]	608	658	

[†] Level of probability for N fertilizer rate effect on carrying capacity (P= 0.0518).

[‡] Level of probability for year effect on carrying capacity (P= 0.0375).

Herbage consumed (HC), a function of intake and stocking rate, was affected by N rate (P=0.0587) but no other factor or interaction. Herbage consumed means across years for the low and high N rate were 202 and 242 kg ha^{-1} d^{-1}. These results reflect the general nature of the N rate effect on carrying capacity.

An N rate by supplement interaction was observed for GAIN (P=0.0072; table 3.12). Interaction occurred because there was a much greater increase in GAIN due to supplementation when pastures received 50 kg N ha^{-1} than when they received 150 kg N ha^{-1} (Table 3.16). Unsupplemented heifers gained nearly 200 kg ha^{-1} more when pastures were fertilized with 150 compared to 50 kg N ha^{-1}. Using PDIFF to compare supplements within N fertilizer rates shows greater GAIN, an average of 90 kg ha^{-1}, for heifers receiving the CUUP supplement over those receiving CU at both N fertilizer rates (Table 3.16).

Table 3.16. Interaction of pasture N fertilizer rate and supplement treatment on gain per hectare of yearling heifers grazing limpograss. Data are means across the 1992 and 1993 grazing seasons (n=4).

	Supplement[†]		
N rate	NONE	CU	CUUP
kg ha^{-1}	----------	kg ha^{-1}	----------
50	37[‡]	242	332
150	232	246	337

Results of significance tests (P value) of planned comparisons among treatment means:

50NONE vs. 150NONE	0.0007
50 vs. 150	0.0084[§]
CU vs. CUUP	0.0059[¶]
50CU + 50CUUP vs. 50NONE	0.0001
150CU + 150CUUP vs. 150NONE	0.0669

[†] NONE, CU, and CUUP are no supplement, corn + urea, and corn + urea + rumen undegradable protein, respectively.

[‡] Standard error of an interaction mean equals 23.0.

[§] Main effect comparison was not used because of interaction.

[¶] Due to interaction, PDIFF was used to compare supplement treatments

50CU vs. 50CUUP (P= 0.0260)
150CU vs. 150CUUP (P= 0.0252)
50NONE vs. 50CU (P= 0.0005)
150NONE vs. 150CU (P= 0.6657)
150NONE vs. 150CUUP (P= 0.0142)

Animal Responses and Herbage Nutritive Value by Period Within Years

Previous studies with limpograss have demonstrated a marked seasonality of pasture and animal performance (Rusland et al., 1988; Holderbaum et al., 1991). For that reason and to provide greater perspective on the relationships between pasture characteristics and animal responses, period data within years will be presented. So that the effect of supplement does not confound these relationships, only data from the 50NONE and 150NONE treatments will be considered in this section.

There was a significant response of herbage CP to the application of the higher level of N fertilization in Period 2 of 1992 (Fig. 3.1) and in all grazing periods of 1993 (Fig. 3.2). With the exception of Period 3 in 1992 CP concentration in the 150NONE treatment was above 73 g kg^{-1}. The worst scenario occurred at the low level of N fertilization during Period 3, when CP concentrations were 47 and 49 g kg^{-1} (1992 and 1993). According to Hodgson (1990), CP concentrations below 625 g kg^{-1} of DM reduce herbage intake and digestion.

When analyzed by period, handplucked herbage IVDOM differed between 50NONE and 150NONE treatments only in Period 3 of 1993 (Figs. 3.3 and 3.4). Herbage IVDOM concentration tended to decrease from Period 1 to Period 3, with the lowest values (480 to 498 g kg^{-1}) measured during September in both years.

Higher N fertilization rate reduced herbage DOM/CP ratio from a range of 8.7 to 10.9 (low N rate) to a range of 7.1 to 8.6 (high N rate). The DOM/CP ratio was lower for the high N rate treatment in all periods of 1993 and in Period 2 of 1992 (Figs. 3.5 and 3.6).

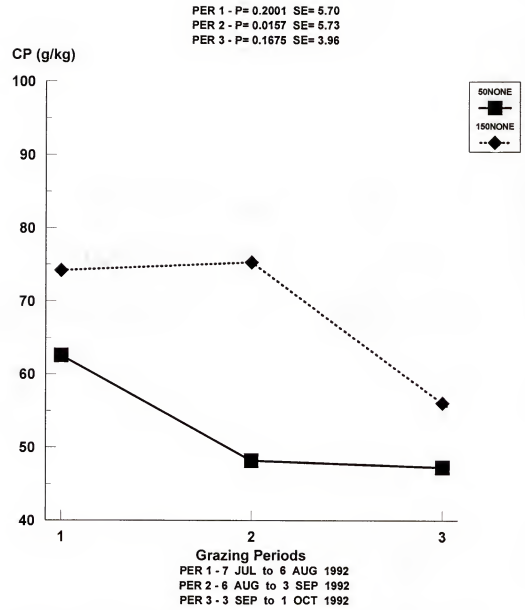

Fig. 3.1. Herbage crude protein (CP) concentration by grazing period on unsupplemented treatments in 1992.

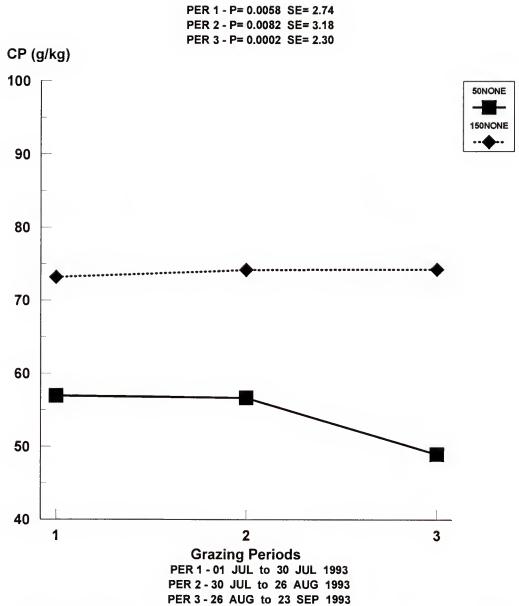

Fig. 3.2. Herbage crude protein (CP) concentration by grazing period on unsupplemented treatments in 1993.

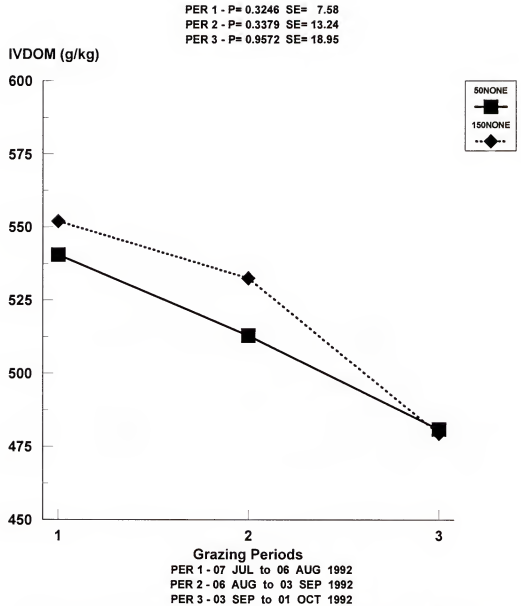

Fig. 3.3. Herbage in-vitro digestible organic matter (IVDOM) concentration by grazing period on unsupplemented treatments in 1992.

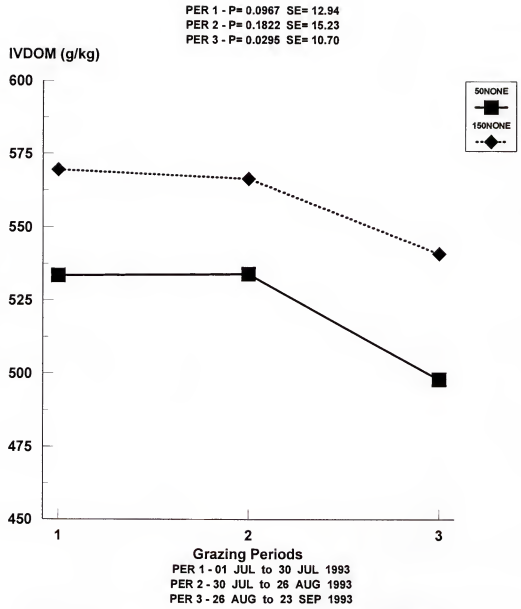

Fig. 3.4. Herbage in-vitro digestible organic matter (IVDOM) concentration by grazing period on unsupplemented treatments in 1993.

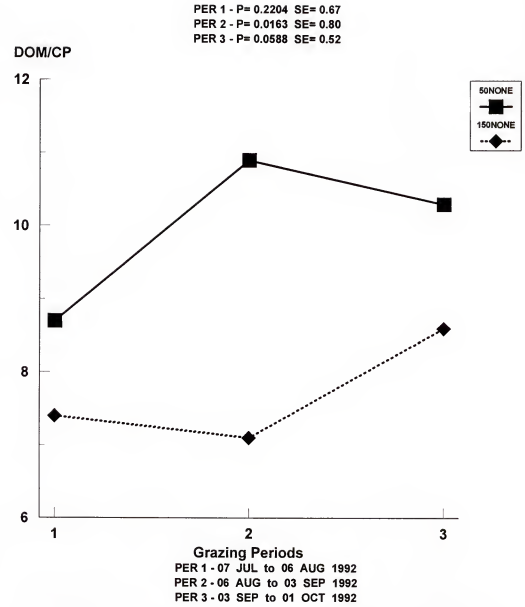

Fig. 3.5. Herbage digestible organic matter (DOM)/crude protein (CP) ratio by grazing period on unsupplemented treatments in 1992.

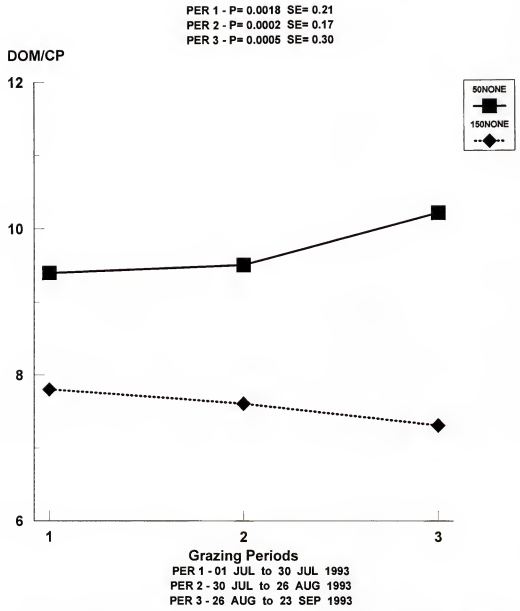

Fig. 3.6. Herbage digestible organic matter (DOM)/crude protein(CP) ratio by grazing period on unsupplemented treatments in 1993.

Because variation associated with measurement of short-term animal weight changes is high (Matches, 1969), no differences in ADG were detected between 50NONE and 150NONE within periods (Figs. 3.7 and 3.8). Trends generally favored the heifers grazing the pastures fertilized with the high N rate. Among periods, heifers on the 150NONE treatment achieved greatest gain in Period 2 in both years. Gains were 0.57 and 0.66 kg d^{-1}, respectively in 1992 and 1993.

The 150NONE treatment increased PUN concentrations above those of 50NONE in most periods in both years (Figs. 3.9 and 3.10). Very low PUN values (range from 2.6 to 3.5 mg $[100\ mL]^{-1}$) were measured for heifers grazing the 50NONE treatment in 1992. In general PUN concentrations were higher for the unsupplemented treatments in 1993 than 1992.

Discussion

The discussion section will focus on the hypotheses that formed the basis for the research. First, consideration will be given to hypotheses about the effect of N fertilizer rate on herbage nutritive value and animal responses. Because there were N rate by supplement interactions for many responses, this first section of the discussion will deal only with the 50NONE and 150NONE treatments. Secondly, hypotheses addressing the effect of supplementation and the N fertilizer rate by supplement interaction will be discussed. In each of these two main sections, data will be discussed in three subgroups of response variables including herbage nutritive value, heifer ADG and PUN, and heifer CC and GAIN.

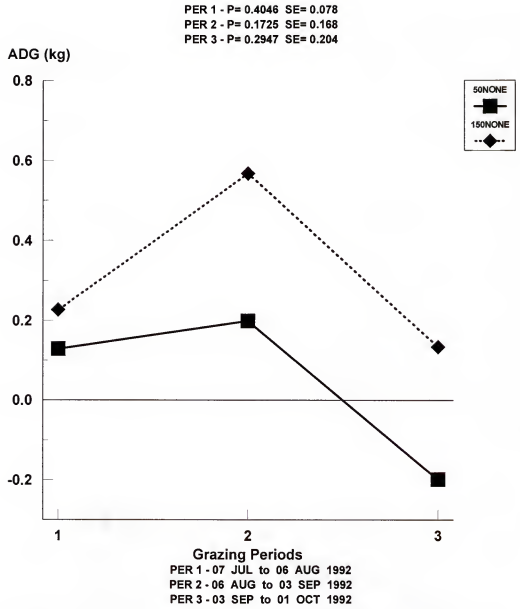

Fig. 3.7. Heifer average daily gain (ADG) by grazing period on unsupplemented treatments in 1992.

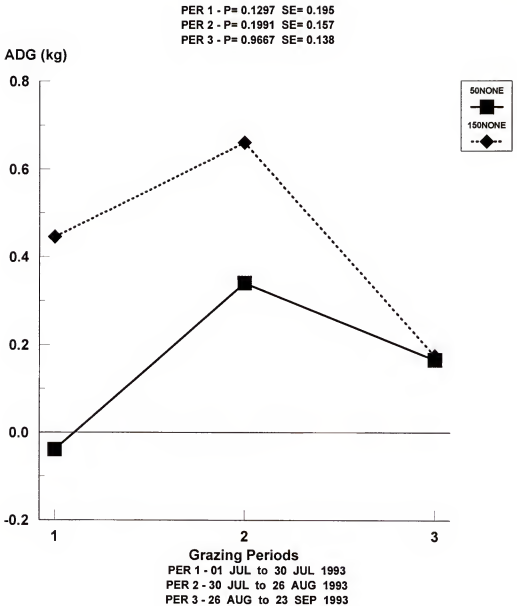

Fig. 3.8. Heifer average daily gain (ADG) by grazing period on unsupplemented treatments in 1993.

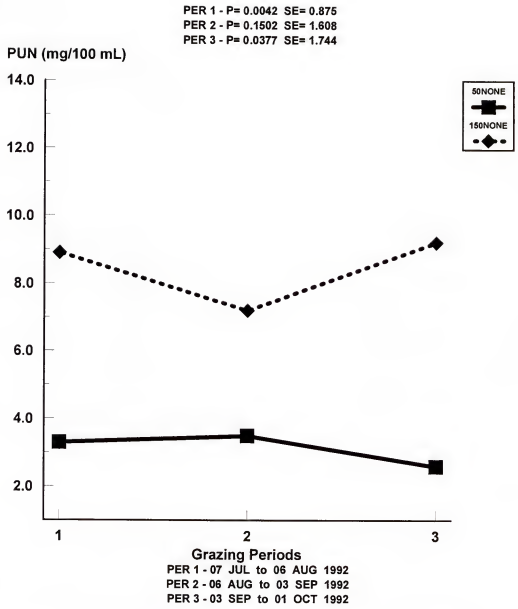

Fig. 3.9. Heifer plasma urea nitrogen (PUN) by grazing period on unsupplemented treatments in 1992.

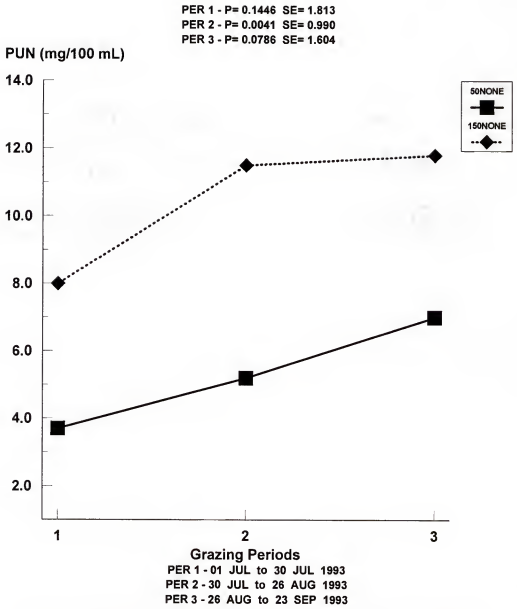

Fig. 3.10. Heifer plasma urea nitrogen (PUN) by grazing period on unsupplemented treatments in 1993.

Effect of N Fertilizer Rate on Herbage Nutritive Value and Animal Responses

Herbage nutritive value

The first group of hypotheses to be tested in this study were related to the effect of N fertilization on herbage nutritive value. Based on the literature, it was hypothesized that increasing limpograss pasture N fertilization rate would increase herbage CP concentration (Christiansen et al., 1988), have little effect on herbage IVDOM (Wilson, 1982), and decrease herbage DOM/CP ratio.

Results from the current study showed that higher pasture N fertilizer rates did increase total herbage and plant-part CP concentrations. Similar responses have been reported for numerous tropical grasses (Dougherty and Rhykerd, 1985; Humphreys, 1987; Hodgson, 1990). Unlike many experiments in which increasing N rate had little effect on IVDOM (Wilson, 1982; Messman et al., 1991), in this study IVDOM was greater with the higher (544 g kg^{-1}) than the lower N rate (516 g kg^{-1}). Though the higher N rate resulted in herbage with a greater leaf/stem ratio, change in leaf percentage did not explain the IVDOM response. Leaf and stem + sheath fractions had similar IVDOM, varying by only 6 to 12 g kg^{-1}. This response is typical for limpograss. Holderbaum et al. (1991) reported that stem plus sheath fractions in the upper and lower parts of the canopy had similar or greater IVDOM than leaf blade from the same region of a limpograss canopy. One other possible explanation for greater IVDOM with higher N fertilizer rate could be that greater CP concentration of this herbage provided sufficiently more N to rumen microbes causing microbial digestion to be enhanced. In this study, however, greater herbage CP concentration

should not influence in vitro digestibility determinations because the analytical protocol used involves feeding soybean (*Glycine max* L.) meal to the donor animal 1 h before collection of rumen fluid. As a result, the N concentration in the tube should not have been limiting for any of the treatments assessed. Thus, the reason remains unclear for greater IVDOM with higher pasture N rate.

The relationship between herbage IVDOM and CP concentrations expressed as DOM/CP ratio is very important in determining an animal's protein status (Hogan and Weston, 1991; Moore, 1992; Kunkle et al., 1994). Nitrogen fertilization at the higher rate increased herbage CP by an average of 30% (17 g kg^{-1}) over that observed for the lower N rate. In contrast, herbage IVDOM increased only 6.5% (33 g kg^{-1}) for the higher compared to the lower N rate. So, despite that fact that IVDOM increased with higher N rates, it increased to a lesser degree than CP, causing herbage DOM/CP to decrease. This response was linked to an increase in herbage leaf/stem ratio with higher N fertilizer rate. The change in leaf/stem ratio was significant because of the IVDOM and CP concentrations in these plant fractions. Crude protein of leaf blades (mean of 106 g kg^{-1} across N rates) was more than twice that of the stem plus sheath fraction (mean of 45 g kg^{-1} across N rates). Thus, the increase in leaf percentage accounted for an important part of the increase in CP observed with the higher N rate. Similar to limpograss plant-part data reported by Holderbaum et al. (1991), leaf IVDOM in this study was not greater than stem plus sheath IVDOM. Thus, increasing N rate increased leaf proportion in the herbage which had a much greater impact on herbage CP than IVDOM concentration.

Heifer average daily gain and plasma urea N concentration

Additional hypotheses about effects of pasture N fertilization rate stated that increasing N fertilization would increase heifer PUN and ADG. These hypotheses were supported by data from the current experiment. Increasing N rate from 50 to 150 kg ha^{-1} increased heifer PUN from 4.2 to 9.2 mg (100 ml $)^{-1}$ and ADG from 0.06 to 0.36 kg.

These animal responses can be attributed to changes in herbage composition caused by fertilization. One effect of higher N rate was to increase herbage leaf/stem ratio. This may have contributed in part to greater ADG of heifers grazing 150NONE vs. 50NONE. Intake of leaf has been reported to be 42% greater than stem of the same digestibility in 26 comparisons using tropical grasses (Laredo and Minson, 1973 and 1975). Our data suggest, however, that the most important factors driving the ADG and PUN responses were herbage CP concentration and DOM/CP ratio.

Heifers grazing the 50NONE treatment consumed herbage with an average of 53 g CP kg^{-1} and a DOM/CP of 9.7. Increasing N rate to 150 kg ha^{-1} increased CP to 70 g kg^{-1} and decreased DOM/CP ratio to 7.7. When CP concentration of pastures falls below 60 to 80 g kg^{-1} this results in protein deficiencies, depression of appetite, low DM intake, and poor performance (Minson and Milford, 1967). Although CP concentration of herbage from the 150NONE treatment was marginal according to the criteria of Minson and Milford, the higher CP and lower DOM/CP in this forage than in 50NONE forage had a major impact on animal performance. Moore and Kunkle

(1995) has suggested that cattle grazing forages with DOM/CP ratios >7 should respond to N supplementation by increasing forage intake and performance. According to Hammond et al. (1993), PUN concentrations are positively correlated with ruminal ammonia N concentration and are indicative of the energy/protein ratio of the diet. Cattle PUN concentrations of < 8 mg $(100\ ml)^{-1}$ indicate that protein may be limiting performance. Activity of cellulolytic microorganisms is reduced at low levels of ammonia release, and this can be a major problem for low CP, high NDF diets like limpograss herbage (Bates et al., 1988; Moore et al., 1991; Hammond, 1992; Kunkle, 1993; and Hammond et al., 1993).

To put the data from the current study in perspective with earlier work, a summary table was constructed that includes data from Sollenberger et al. (1989), Holderbaum et al. (1991), and the two N-rate treatments from the current study (Table 3.17). Relationships are quite consistent between pasture N fertilizer rate and herbage composition (CP and DOM/CP), between herbage composition (CP and DOM/CP) and animal responses (ADG and PUN), and between PUN and ADG.

As observed in previous studies (Rusland et al., 1988; Sollenberger et al., 1989; Holderbaum et al., 1991), there was pronounced seasonality in heifer ADG on limpograss pasture. Average daily gain was lowest for the 50NONE treatment in the last period of 1992, falling to -0.2 kg d^{-1}. During this period, herbage CP and IVDOM concentrations and heifer PUN concentration were at their lowest levels of the year, and DOM/CP ratio was in excess of 10 (Figs. 3.1, 3.3, and 3.11). In 1993, lowest ADG occurred in Period 1 in conjunction with lowest heifer PUN, but

Period 1 IVDOM and CP concentrations were the highest of the year, and DOM/CP ratio was at its lowest for the year (Figs. 3.2, 3.4, and 3.12). Patterns in ADG across the season generally were similar between pasture N rates despite the effects of greater N rate on herbage characteristics. There was a consistent trend for ADG to be greater for 150NONE than 50NONE, but variation among animals treated the same was large and differences within periods could not be detected.

Heifer carrying capacity and gain per hectare

Applying 150 compared to 50 kg N ha^{-1} resulted in an increase in CC of approximately 100 heifer days ha^{-1} in 1992 when rainfall was near normal. In the drier than normal year of 1993 (58% of 70-yr average rainfall during July through September), the higher N rate promoted an increase in CC of only 37 heifer days ha^{-1}. Gain per hectare was increased six-fold when unsupplemented heifers grazed pastures fertilized with 150 compared to 50 kg N ha^{-1}. The greatest proportion of this increase was due to greater ADG for the higher N rate treatment.

Supplement and N by Supplement Interaction Effects on Herbage and Animal Responses

Herbage nutritive value

Supplement treatments had little effect on herbage nutritive value. There was an N rate x supplement interaction effect on herbage CP concentration, but it occurred primarily because of differences among supplement treatments in magnitude (not direction) of the increase in herbage CP due to use of the higher N fertilizer rate. Herbage IVDOM was greater in pastures where heifers received the CUUP supplement, but there is no apparent explanation for this response. There were

Table 3.17. Summary table of pasture and animal responses for experiments evaluating rotationally stocked, N-fertilized limpograss pastures.

Item	Sollenberger et al., 1989	Holderbaum et al., 1991	Lima unpublished	Lima unpublished
N rate (kg ha^{-1})	180	123	50	150
Rest period (d)	35	35	28	28
Years of study (yr)	3	2	2	2
Days of grazing (d)	168	84	91	91
Herbage CP (g kg^{-1})†	82	67	53	70
Herb. IVDOM (g kg^{-1})†	616	590	516	544
Herbage DOM/CP†	7.5	8.7	9.7	7.7
ADG (kg d^{-1})	0.41	0.29	0.06	0.36
GAIN (kg ha^{-1})	460	168	37	232
PUN (mg 100 mL^{-1})	NA‡	6.0	4.2	9.2

† - Handplucked herbage selected to represent the diet for all experiments.
‡ - Not available.

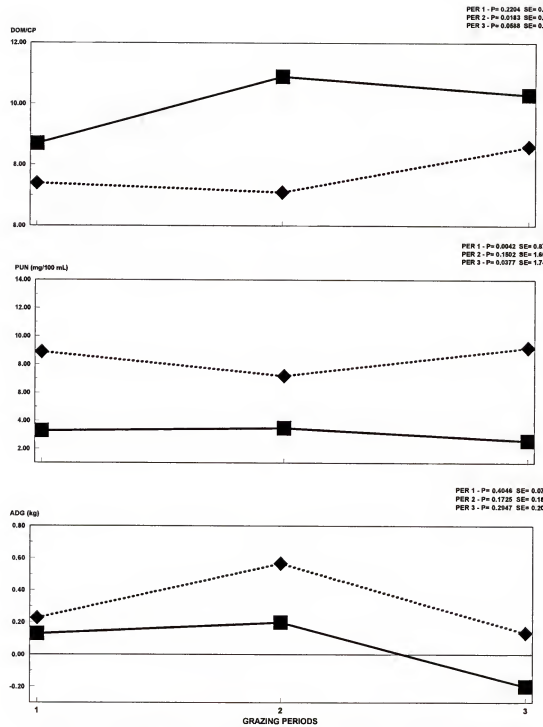

Fig. 3.11. Responses of DOM/CP, PUN, and ADG, by grazing period for the unsupplemented treatments in 1992. Grazing periods (1- 07 Jul. to 06 Aug.; 2- 06 Aug. to 03 Sep.; and 3- 03 Sep. to 01 Oct.). Treatments (■ - 50NONE and ▲ - 150NONE).

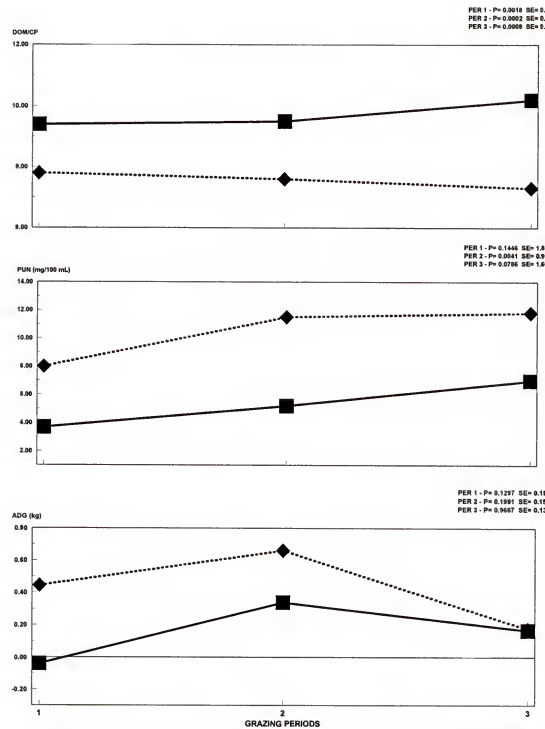

Fig. 3.12. Responses of DOM/CP, PUN, and ADG, by grazing period for the unsupplemented treatments in 1993. Grazing periods (1- 01 Jul. to 30 Jul.; 2- 30 Jul. to 26 Aug.; and 3- 26 Aug. to 23 Sep.). Treatments (■ - 50NONE and ▲ - 150NONE).

interacting effects of supplement and N rate on herbage DOM/CP ratio, with ratio being greater for CUUP than CU when N rate was 50 kg ha^{-1}, but tending to be greater for CU ($P = 0.0792$) than CUUP when N rate was 150 kg ha^{-1}. As with the effect of supplement on IVDOM, there is no apparent explanation for these responses. Leaf/stem ratio was not affected by supplement treatment.

Heifer average daily gain and plasma urea N concentration

Previous studies contributed to development of hypotheses related to effect of rumen undegraded intake protein on heifer performance. Holderbaum et al. (1991) attempted to address the problem of limited dietary CP on limpograss pastures during summer by using corn plus urea (NPN) supplementation. Daily gains of supplemented steers were 80 to 100% greater than those of unsupplemented animals. Holderbaum (1989) stated that during specific times of the grazing season (August to September) supplementation with NPN did not overcome a slump in production observed in supplemented and unsupplemented animals alike. There is disagreement in the literature regarding how efficiently NPN (specifically urea) can be used to meet rumen degradable protein requirements. As a supplement to low quality forages, NPN is considered an inferior N source compared with intact protein (Church, 1991; Owens et al., 1991; Kunkle, 1993b). Data obtained using growing heifers grazing warm-season pastures suggested that inadequate daily intake of UIP limited animal performance even though the grazed forages contained 140 to 180 g CP kg^{-1} (Ellis, 1990). He attributed this low efficiency of protein utilization to extensive ruminal degradation of herbage protein.

The current study was designed to complement the results obtained by Holderbaum et al. (1991) and to explore the effect of addition of UIP to an NPN supplement. The hypothesis to be tested stated that average daily gain of yearling beef heifers grazing limpograss is improved by increasing the amount of rumen UIP in a primarily rumen degradable protein supplement of corn plus urea.

Heifer ADG was affected by an N rate by supplement interaction. Interaction occurred because supplemented heifers outgained those receiving no supplement when N rate was 50 kg ha^{-1} but not when N rate was 150 kg ha^{-1}. Higher rate of N fertilizer increased PUN concentrations, increased herbage CP, and reduced response to the protein supplements. The 150NONE treatment had an average DOM/CP of 7.7 during both years. According to Moore and Kunkle (1995) supplementation of forages with a balanced DOM/CP ratio (<7) decreases intake in proportion to the amount of concentrate fed. Relative to the stated hypothesis, heifers receiving the CUUP supplement did outgain those receiving CU whenever N rate was 50 kg N ha^{-1} but not when N rate was 150 kg ha^{-1}. It remains unclear why ADG for the 150CUUP treatment did not exceed those for the 150CU. One possible explanation could be linked with the higher CP concentration on the pastures fertilized with 150 kg N ha^{-1}. Protein of warm-season grasses is considered more slowly degraded than cool-season grasses (Akin, 1989), a characteristic that can provide a greater proportion of protein for utilization in small intestines (Karges et al., 1992). The 30% increase in herbage CP concentration provided by the higher N fertilizer rate could possibly have increased the passage of herbage UIP to the intestines and limited the response to the

150CUUP treatment. The better performance of the 50CUUP treatment compared with 50CU indicates that microbial protein synthesis plus UIP provided by the corn + urea supplement and limpograss herbage may not be able to satisfy the metabolic protein requirement of the heifers. Owens et al. (1991) suggested that supplemental rumen undegraded protein, by evading rumen fermentation can directly increase the intestinal supply of amino acids and glucogenic compounds. They reported that CP supplement often increases performance of ruminants grazing low quality forages through an increased intake of digestible nutrients. Reduction in intake of animals grazing forages deficient in CP seems to be caused by low availability of amino acids rather than by digestibility per se (Minson, 1982; Nolan and Leng, 1989).

Brown and Pitman (1991) reported low absolute quantities of ruminal soluble and degradable N for limpograss, and the low degradable N may limit microbial protein synthesis in the rumen. It is likely as evidenced by DOM/CP ratio and PUN levels that the CU supplement by its degradable characteristics could have addressed the ruminal protein deficiency, but was not able to meet the demand for intestinal protein. The superior performance of the CUUP treatment may be linked with the presence of digested undegraded protein (DUP) at the intestinal level. Data reported by Egan (1976), suggested that voluntary intake of forage may not be maximized until levels of intestinally absorbed protein exceeds 20% of digested organic matter. The potential for increasing gains from warm-season pastures through utilization of DUP supplements is supported by Ellis (1990). He associates the efficiency of DUP supplements with a possibly better amino acid balance improving metabolic utilization

of absorbed protein. He also recommended restricted amounts of daily supplements (< 1 kg d^{-1}, for a 250 kg calf), in order to maximize the supplementary effect and minimize substitutive effects.

The lack of an ADG response to supplements at the higher N rate can also be explained by looking at heifer PUN data. The PUN response and its relationship to ADG are in agreement with Hammond et al. (1993). They reported that there is a transition PUN range (from 9 to 12 mg 100 ml^{-1}) below which ADG response to protein supplementation is greater and above which ADG response is lesser. Plasma urea N concentrations were 4.2 for 50NONE, 9.2 for 150NONE, and above 15 for all supplemented treatments.

It seems clear that a better understanding of the relationship between protein supplements and substitutive or associative effects on animals grazing low quality warm-season grasses requires more appropriate and precise measures of herbage intake than the ones performed in the present research.

Heifer carrying capacity and gain per hectare

Gain per hectare increased greatly due to supplementation when pastures were fertilized with 50 kg N ha^{-1} but the increase was not as great when pastures received 150 kg N ha^{-1}. This difference in response accounts for the N by supplement interaction. Within an N rate, both 50NONE and 150NONE had lower GAIN than the average of the two supplemented treatments. The GAIN of heifers supplemented with additional UIP (335 kg ha^{-1}) was superior to corn + urea supplement at low

(P=0.0260) and high (P=0.0252) levels of N rate. The CUUP treatment promoted an average increase in GAIN over CU of 90 kg ha^{-1}.

Economics of Meeting Crude Protein Demand of Replacement Heifers

Maintenance demands of breeding and replacement heifers constitute a substantial proportion of the nutrient demand of the whole herd in meat production enterprises (Hodgson, 1990). He suggested that breeding efficiency and prolificacy of those animals can directly affect the efficiency of the whole system. Kunkle (1993b) evaluated several strategies for cost effective supplementation of beef cattle. He indicated that protein supplementation was the second priority after minerals, and that protein supplementation generally was more cost effective than energy supplements. He also stated that feeding 0.09 to 0.14 kg d^{-1} of a protein supplement costs 5 to 10 cents and increased TDN intake of the cow by 0.9 kg or more.

In the present experiment, cost (calculated as material for N fertilizer and supplement) per kg of gain, above that observed on 50NONE, was less for 150 NONE ($0.39/kg) and 50CUUP ($0.41) than all other treatments. These results were similar to those reported by Holderbaum et al. (1991) when using a corn + urea supplement ($0.44/kg). These costs can be compared with the extra gain acquired due to protein supplements ($0.73/kg; including labor and equipment) in Texas (Ellis and Hill, 1994, Personal communication). They used growing calves grazing heavily fertilized and intensively managed bermudagrass (*Cynodon dactylon* L.) pastures in Texas. Use of protein supplementation for nursing calves on cooperating ranches in Florida, Kunkle et al. (1993) found a $6/head return at weaning. Calf gains were

increased 0.14 kg d^{-1}, close to 1 kg of protein was required for each 0.45 kg of additional gain, with a total cost of $1 for each extra kg (including labor and equipment) obtained over unsupplemented animals.

Summary and Conclusions

Raising beef replacement heifers efficiently represents a critical segment of integrated cow-calf programs in Florida. The appropriate balance between forage management practices to improve herd nutrition and supplementation can directly affect the breeding efficiency, prolificacy, genetic improvement of the herd, and consequently future profitability of the system. According to Kunkle and Sand (1993), "Several Florida cattlemen are successfully and profitably calving heifers at two years of age but this is a challenge to the best managers." Protein and energy supplements are often required even in summer because perennial pastures or hay in Florida do not have adequate nutrient composition or quality to meet the nutrient demands of weaned calves, developing heifers, or cows approaching the breeding season (Moore et al., 1991).

To aid in development of cost-efficient replacement heifer programs, a 2-yr animal performance study was conducted. The objectives of the study were to evaluate the effects of N fertilization rate of limpograss pastures and heifer supplementation with different N sources as management alternatives in summer grazing systems for yearling beef heifers.

There was no effect of N rate on ADG of supplemented heifers, but ADG of unsupplemented heifers was less on pastures fertilized with 50 kg N ha^{-1} than on

pastures receiving 150 kg. Increasing N fertilizer rate increased CC, increased herbage CP concentration, increased heifer PUN, and reduced heifer ADG response to supplements. These responses corroborate work by Hammond et al. (1993) who reported that there is a transition range in PUN (from 9 to 12 mg [100 ml]$^{-1}$) below which ADG response to protein supplements is greater and above which ADG response is lesser. Also these data are in agreement with the findings of Moore and Kunkle (1992) that supplements should increase animal performance when forage DOM/CP ratios are >7. They also suggested that supplementing forages with a more nearly balanced DOM/CP ratio (<7) will decrease intake in proportion to the amount of concentrate fed. Even without intake data to confirm this assumption it may explain the lower response to supplements on pastures fertilized with 150 kg N ha^{-1}. Average daily gains of heifers grazing the 50CUUP treatment were superior to those on 50CU, but there was no difference between 150CU and 150CUUP. The better performance of the 50CUUP treatment compared with 50CU indicated that microbial protein synthesis plus UIP provided by the corn + urea supplement and limpograss herbage may not be able to satisfy the metabolic protein requirements of the heifers.

Neither N fertilization nor supplementation were able to avoid a decrease in ADG during late August to early October (summer-slump). Even without a reliable measure of limpograss herbage intake, the evidence suggests that low ADG for the 50NONE treatment is probably related to low herbage CP and IVDOM concentrations, high DOM/CP ratio, and low PUN values. These same responses,

however, were not enough to explain why the 150NONE and supplemented treatments did not overcome the decrease in ADG during the summer-slump period. Improved methodologies for measuring herbage intake on pastures and for studying herbage nutrient utilization seem necessary to address this particular problem.

Normally regarded as the response of greatest economic importance, GAIN was clearly favored by fertilization and supplementation. The 150NONE treatment achieved an advantage of almost 200 kg ha^{-1} over 50NONE. The GAIN for treatments receiving additional UIP (50CUUP and 150CUUP) was superior to all others.

Based on the results of the present research some conclusions can be drawn.

i) Increasing N fertilization from 50 to 150 kg ha^{-1} increased herbage CP concentration, decreased DOM/CP ratio, increased heifer PUN concentration, and increased heifer ADG and GAIN.

ii) Heifer ADG increased greatly when supplements were fed to animals grazing limpograss fertilized with 50 kg N ha^{-1}, but response to supplementation was reduced when pastures received 150 kg N ha^{-1}.

iii) Heifers receiving the CUUP supplement had a better overall ADG and GAIN than those receiving CU or NONE. This response to UIP indicated that neither protein synthesized in the rumen nor UIP from CU or limpograss herbage were enough to meet the heifers protein requirements.

iv) Analysis of cost per kg of additional gain (above that for 50NONE) suggested advantages for the treatments 50CUUP ($0.41/kg) and 150NONE ($0.39/kg).

v) We concluded that both N fertilization and supplementation can be used to overcome N deficiencies of heifers grazing limpograss.

vi) Further research is needed to test different sources and levels of UIP and energy, particularly targeting the summer slump period.

CHAPTER 4
NITROGEN CONCENTRATION AND FRACTIONATION OF WARM-SEASON GRASSES IN FLORIDA

Introduction

Forages provide the majority of nutrients to the 1.2 billion dollar livestock industry in Florida. Recent estimations suggest that there are approximately 1.54 million ha of planted perennial pastures in Florida, 3.65 million ha of range, and 0.5 million ha of planted temporary pastures (C.G. Chambliss, 1995 personal communication; R.S Kalmbacher, 1995, personal communication). Of the planted perennial pasture species 'Pensacola' bahiagrass (*Paspalum notatum* Flugge) is used on more land area than any other, and covers an estimated 1.0 million ha (Chambliss and Sollenberger, 1991). Other important planted warm-season grasses utilized to varying degrees in Florida are bermudagrass (*Cynodon dactylon* L.), limpograss [*Hemarthria altissima* (Poir.) & C. E. Hubb.], stargrass (*Cynodon nlemfuensis* Vanderyst) and digitgrass (*Digitaria eriantha* Steud.).

There are large seasonal variations in quality and quantity of pasture grasses, and there are times when these grasses do not meet the maintenance and production needs of particular ruminant livestock classes. Because of that, energy and protein supplementation are common practices in the state (Pate, 1991; Kunkle, 1993b;

Kunkle et al., 1993).

To determine the type and amount of supplement needed in grazing systems requires knowledge of the contribution of the basal diet to the requirement of the host (Ørskov, 1976). Besides animal and ruminal microbial requirements, Ellis (1990) listed the degradable and undegradable protein in the forage, available energy intake from forage, and interactions between the forage and supplement as required information before a supplement can be formulated for grazing cattle. In discussing the unknowns involved in predicting limiting nutrients in grazing situations, Klopfenstein (1993) stated "The largest unknown is the composition of the protein in the grass." He emphasized that the greatest need is to obtain more information about the forage protein fractions. Hart and Leibholz (1990) agree and reported that several estimates have been made of the degradability of protein supplements, but there is little information on the degradability of protein from grasses in the rumen.

Most new protein systems take into account the degradability of dietary protein and synthesis of microbial protein in the rumen to define the whole animal requirements for dietary protein (Chalupa and Sniffen, 1991). Describing methods of assessing forage protein quality, Broderick (1994) suggested that ideally they should describe forage contribution to bacterial crude protein (BCP) and undegradable intake protein (UIP) to meet animals' absorbed protein requirements. The relative solubility and degradability of dietary N compounds are important determinants of efficiency of microbial protein synthesis and economics of feeding animals with a high nutrient

requirement (Krishnamoorthy et al., 1991; Reid, 1994). The shortage of information in this area of study is more pronounced when considering warm-season grasses.

This experiment was designed as a follow-up study to the animal performance trial reported in Chapter 3. The general objective for this work was to more completely describe the N fractions in 'Floralta' limpograss and two other warm-season grasses ('Tifton-85' bermudagrass and Pensacola bahiagrass) under different management treatments. The objectives of the trial were: i) To evaluate the influence of N fertilizer rates, age of regrowth, and season on herbage N fractions of three warm-season grasses; ii) to test in-vitro analysis to assess herbage protein solubility and degradability; and iii) to use data from this study to aid in explaining animal responses observed in the grazing performance trial (Chapter 3).

Materials and Methods

The experiment was conducted at the Beef Research Unit of University of Florida located northeast of Gainesville, Florida. The experimental period was from May to October of 1994.

The design used was completely randomized with treatments replicated three times and arranged as a split-plot experiment. Main plots were combinations of three grass species (Floralta limpograss, Tifton-85 bermudagrass, and Pensacola bahiagrass), with two seasons of growth (early and late summer). Subplots were combinations of three ages of regrowth (4, 6, and 8 wk), and two N fertilizer rates (17 and 50 kg N ha^{-1}). The two N fertilizer rates were chosen because they represent

the amount applied at each of the three N applications utilized in the animal performance trial (Chapter 3). Plot size was 2 m^2.

Soils at the research site were moderately to poorly drained flatwood types. They included sandy Spodosols of low fertility, belonging primarily to the Pomona and Smyrna series.

Plots were staged by clipping bahiagrass to a 5-cm stubble height and limpograss and bermudagrass to a 10-cm stubble height. Age of regrowth treatments were staged at 1-wk intervals as shown in Table 4.1. By staggering both staging and sampling dates, the mid-period of regrowth was the same for all age treatments. Season 2 plots were pre-staged 4 wk prior to their staging cut so that regrowth after staging represented normal growth rates for that time of the year. On 24 May, all plots received an application of 0-10-20 at a rate of 448 kg ha^{-1}. The two N fertilizer rates were applied as ammonium nitrate just after the staging cut.

Sampling was performed at scheduled times (Table 4.1) in July and September of 1994. Two 0.25-m^2 sampling units were clipped at the same stubble heights used at staging. One of the two samples was immediately put on ice in a cooler. Samples were then stored in a freezer (-10° C) until being freeze dried and stored for subsequent analysis. Samples were ground through a Christie Norris hammer mill fitted with a 1-mm screen. The second sample was separated manually into leaf blade and stem plus sheath (true stem, leaf sheath, and the peduncle) fractions and dried at 60°C to determine leaf percentage and total DM production.

Table 4.1. Schedule for staging and harvesting.

25	May	Stage 8-wk Season 1
01	June	Stage 6-wk Season 1
08	June	Stage 4-wk Season 1
23	June	Pre-stage 8-wk Season 2
30	June	Pre-stage 6-wk Season 2
06	July	Harvest 4-wk Season 1
07	July	Pre-stage 4-wk Season 2
13	July	Harvest 6-wk Season 1
20	July	Harvest 8-wk Season 1
21	July	Stage 8-wk Season 2
28	July	Stage 6-wk Season 2
04	Aug.	Stage 4-wk Season 2
01	Sep.	Harvest 4-wk Season 2
08	Sep.	Harvest 6-wk Season 2
15	Sep.	Harvest 8-wk Season 2

Samples were analyzed for laboratory dry matter (DM) and organic matter (OM) according to A.O.A.C. (1984) procedures. In-vitro digestible organic matter (IVDOM) was determined using a modified two-stage procedure (Moore and Mott, 1974). A modified aluminum block digestion procedure (Gallaher et al., 1975) and semi-automated colorimetry (Hambleton, 1977) were used for all N determinations. Crude protein (CP) was calculated as N x 6.25. The N fractions analyzed were nonprotein N (NPN) and in-vitro readily soluble true protein after 2 h of incubation

(RSP) (Neutze et al., 1993), acid detergent insoluble N (ADIN), and neutral detergent insoluble N (NDIN) (Goering and Van Soest, 1970).

Nonprotein N (NPN) was analyzed by a modification of the inhibitor in vitro (IIV) method of Broderick (1987), and was quantified as the proportion of the buffer + inoculum soluble N (degradation at 0-time) which was not precipitated by trichloroacetic acid. In this method feed samples are incubated in rumen fluid containing inhibitors of microbial uptake of ammonia and amino acids. Thus, products of protein degradation can be measured without interference from microbial assimilation of some of these breakdown products. The inhibitors employed were hydrazine sulfate and chloramphenicol which prevent, respectively, ammonia and amino acid incorporation for microbial growth. The modification of the Broderick (1987) procedure included an estimation of the degraded protein fraction by non-trichloroacetic acid precipitable N rather than the sum of alpha-amino N and ammonia N as in the original methodology (Neutze et al., 1993).

A preliminary trial was conducted using different incubation times (0, 2, 4, 6, and 12 h) for samples of the three forage species. Samples were run in duplicate within a run and with three replications of the run in different days in order to account for rumen inoculum variability. This trial corroborated use of the incubation period of 2-h recommended for analysis of forage proteins by the U.S. Forage Research Center, USDA-ARS, Madison, WI, (G.A. Broderick, 1994, personal communication), because there was no significant increase in N degradation after 2 h. Since many samples had low CP concentrations, the preliminary trial was also

important to determine appropriate size of samples and all dilutions necessary for analysis of N.

Whole rumen contents were obtained from a rumen-cannulated non-lactating dairy cow fed ad libitum good quality bermudagrass hay and provided with a daily supplement of 900 g of soybean meal, plus free-choice complete mineral mixture. A thermal jug with CO_2 was used to transport the rumen contents from the barn to the lab. Enough was poured into the jug through two layers of cheesecloth and squeezed to give 1.5 L of strained ruminal fluid. The solids inside the cheesecloth were also put into a container that had been gassed with CO_2. The material was transported immediately to the laboratory. The strained ruminal fluid was enriched with particle-associated microbes by washing the solid residue four times with a total volume of McDougall's buffer (McDougall, 1948) equal to the volume of strained fluid to be utilized for the inoculum preparation (1.0 L); the buffer washes were strained through eight layers of cheesecloth and added to the strained fluid. Then the strained fluid plus buffer with solids were mixed and filtered again through eight layers of cheesecloth. Oxygen-free CO_2 was used to purge the vessels in the process of washing rumen solids and in preparation of McDougall's buffer. The strained fluid plus buffer extract was warmed to 39°C in a water bath, then appropriate reagent volumes (Table 4.2) were added to the inoculum 5 min before starting the incubation. Maltose solution was made fresh daily, hydrazine sulfate and chloramphenicol were weighed at the required amounts the day before the incubation.

Table 4.2. Composition of in-vitro inoculum and reagent concentrations in the final medium.

Component	Inoculum concentration (amount/L)	Final medium concentration
Strained rumen fluid	450 mL	300 ml/L
Buffer extract of rumen solids	450 mL	300 ml/L
McDougall's buffer	0	400 ml/L
2-Mercaptoethanol †	234 mg	2.0 mM
Maltose solution (100 mg/mL) †	50 mL	3.3 mg/mL
Hydrazine solution (60 mM) †	25 mL	1.0 mM
Chloramphenicol solution (1.80 mg/mL) †	25 mL	30.0 μg/mL

† Maltose, hydrazine sulfate and chloramphenicol solutions were prepared in McDougall's buffer. Reagents were added to the inoculum in the order: 2-mercaptoethanol, maltose, hydrazine sulfate, and chloramphenicol.
Source: Broderick (1987).

Each forage sample was run in duplicate at 0 and 2 h in two separate incubations to account for variability of activity in ruminal inocula. Four blank tubes (two placed before and two placed at the end) containing all components except added forage protein were included for each 30 forage sample tubes. Six standards of known degradabilities (casein, alfalfa hay, two of soybean meal, and two forages from the preliminary trial) were included in each incubation.

Enough material of each sample was weighed the day before incubation to provide 3.75 mg N in each incubation tube (50 mL in vitro tubes). All the open tubes were covered with cheesecloth overnight. On the incubation day each sample was soaked in 5 ml of McDougall's buffer at 39°C (ungassed). After dosing the

samples they were put into a shaking water bath (39°C and 75-100 cycles per min), covered with plastic wrap to minimize evaporation, and left for 45 min until ready to incubate.

The incubation began by adding 10 ml of 39°C ruminal inoculum mixture to the tubes with samples. Tubes were then flushed with CO_2, capped with Bunsen valves, and returned to the shaking water bath. The final incubation mixture was 15 mL with 1 vol. buffer (samples soaked at 5 mL per tube) and 2 vol. of the inoculum (10 mL per tube in a 1:1 strained fluid:buffer ratio), so the final concentration was 0.250 mg feed N mL^{-1}. Trichloroacetic acid (1.25 mL of a 65% wt/vol trichloroacetic acid to give a 5% trichloroacetic acid final concentration) was added to the 0-time tubes a few minutes before adding the inoculum. In that way, trichloroacetic acid was already present at dosing and the microbes were killed thus precluding microbial protein degradation.

After dosing with inoculum, the tubes were placed in an ice bath to help stop degradation, as well as to minimize foaming. To stop microbial activity at a 2-h incubation time, the procedure used was the same as that for 0-time, except that trichloroacetic acid was added to the whole inoculum. After stopping protein degradation, both 0-time and 2-h incubations were kept on ice for 1 h then a 10-mL aliquot was transferred to a 12 x 75 mm disposable centrifuge tube and centrifuged for 15 min (15300 g, 4°C). A supernatant aliquot of 7 mL was taken from each tube and stored at 4°C until analyzed for total N. For all N determinations, supernatant samples (7 mL) were digested using a modification of the aluminum block procedure

digestion of Gallaher et al. (1975). Instead of diluting the digestate to 75 mL it was diluted to 25 mL so that peaks could be read when ammonia in the digestate (aliquot of 3 ml) was determined by the semi-automated colorimetry method of Hambleton (1977).

The extent of feed protein degraded at 0-time and 2 h were estimated as the quantity of supernatant N present in the incubation tube as a proportion of total N added. Three fractions were calculated using the results of N degradation at 0-time, readily soluble true protein (RSP) at 2-h incubation, total N added, and acid detergent insoluble N (ADIN). Nonprotein N fraction is normally equivalent to fraction A on degradation protein models (Broderick, 1994). The second fraction (RSP) is equivalent to the rapidly degraded true protein fraction (B1) described by Sniffen et al. (1992). That fraction was calculate as degraded N at 2 h minus the degraded N at 0-time. A third fraction was labelled as in-vitro slowly soluble protein (SSP) after 2 h of incubation. It was calculated as total N added at the beginning of incubation minus total N degraded at 2 h (NPN + RSP) minus ADIN. This third fraction (SSP) involves slowly soluble and insoluble protein and is comparable to the sum of the fractions B2 (intermediate degraded protein) and B3 (slowly degraded protein) described by Sniffen et al. (1992).

Determinations of acid detergent fiber (ADF), ADIN, neutral detergent fiber (NDF), and NDIN were performed according to Goering and Van Soest (1970) procedures. The N fractions were measured as the N remaining after NDF and ADF refluxing. Sample weight for ADIN and NDIN determinations were 2 and 1.5 g,

respectively. As a result of the high OM concentration of the ADF and NDF residues, the standard Kjeldahl procedure (A.O.A.C, 1984) was modified to analyze NDIN and ADIN. To promote a slower breakdown of the OM, before adding the hydrogen peroxide (H_2O_2 at 30%) the samples were soaked with 30 mL instead of 20 mL of H_2SO_4 and swirled 2 or 3 times until the filter paper was completely disintegrated. Samples were kept in sulfuric acid for 1 h and then put into the block digestor for 30 min with only sulfuric acid. After that, they were removed from the block to cool for another 30 min. Then H_2O_2 was added in 1 mL increments up to a total amount of 8 mL, or until contents of the tube started turning brown or yellow. After boiling subsided, the tubes were returned to the block digestor for approximately 1 h or until complete digestion was obtained (clear liquid). All the other steps were the same as in the original procedure (Hambleton, 1977) up to the collection of the aliquot (3 ml) for determination of ammonia in the digestate. All the N fractions will be reported as g kg^{-1} DM or g kg^{-1} of total N.

Data were analyzed using analysis of variance in PROC GLM (general Linear Models Procedure) of the Statistical Analysis System (SAS; SAS Institute Inc., 1989). Comparisons among forage species were made using the F-protected LSD test ($P \leq 0.05$). Effects of age of regrowth ($P \leq 0.05$) were assessed using orthogonal polynomial contrasts. Season and N rate comparisons were made using the F test because there were only two levels of these factors. When there were three- or four-way interactions involving season, data were analyzed by season, and interaction or main effects are described within season.

Results and Discussion

Herbage In-Vitro Digestible Organic Matter, Neutral Detergent Fiber, and Leaf Percentage

There was a species by season by age interaction for IVDOM (Table 4.3). In both seasons there was a forage species by age interaction ($P=0.0002$ and $P=0.0011$) (Tables 4.4 and 4.5) and in late summer there was an interaction ($P=0.0040$) of N rate and age of regrowth (Table 4.6). The species by age interaction in early summer occurred because limpograss and bermudagrass IVDOM did not differ at 4 and 6 wk of regrowth, but limpograss had highest IVDOM at 8 wk. In late summer, limpograss IVDOM was highest at both 6- and 8-wk regrowth intervals. Relatively slow rates of IVDOM decline with increasing maturity is a well know positive characteristic of limpograss in Florida (Sollenberger et al., 1988; Quesenberry, 1993). Tifton-85 bermudagrass IVDOM results are comparable with values reported by Sollenberger et al. (1995) and Hill et al. (1995), with the exception of low values at 6- and 8-wk regrowth intervals during the late-summer season. During that period several plots of bermudagrass were affected by a fungal disease (*Helminthosporium* spp.) that promoted leaf senescence, and reduced the vigor and rate of regrowth of the plants. Bahiagrass generally had the lowest IVDOM. These data are similar to those reported by Sollenberger et al. (1988) and Moore (1992). The interaction of age by N occurred because IVDOM was higher for the higher N fertilizer rate only when age of regrowth was 4-wk (Table 4.6). There was no effect on IVDOM when age was 6 or 8 wk.

Table 4.3. Levels of probability for effects of species, season, age of regrowth, N, and their interactions on herbage in-vitro digestible organic matter (IVDOM), neutral detergent fiber (NDF), leaf percentage of total dry matter (LEAF), nitrogen concentration (N), neutral detergent insoluble N (NDIN), and acid detergent insoluble N (ADIN).

EFFECT	IVDOM	NDF	LEAF	N	NDIN†	NDIN‡	ADIN†	ADIN‡
SPECIES (SP)	0.0001	0.0001	0.0001	0.0001	0.0037	0.0001	0.0001	0.0001
SEASON	0.0001	0.0007	0.0001	0.0001	0.8204	0.0001	0.0019	0.0001
SP*SEASON	0.0001	0.3345	0.0001	0.0005	0.1679	0.0001	0.0001	0.0001
AGE	0.0001	0.0001	0.0001	0.0001	0.6965	0.0001	0.0001	0.2119
N	0.2321	0.0159	0.8945	0.0001	0.0378	0.0006	0.0193	0.0001
SP*AGE	0.0001	0.0170	0.0005	0.6775	0.0620	0.2127	0.0008	0.0104
SEASON*AGE	0.0601	0.0059	0.4088	0.0616	0.5586	0.0232	0.1933	0.1843
AGE*N	0.0017	0.0104	0.5505	0.4920	0.4507	0.4817	0.9346	0.9211
SP*N	0.6364	0.7779	0.4258	0.6262	0.0782	0.0035	0.0177	0.0074
SEASON*N	0.0057	0.2695	0.4400	0.4008	0.6065	0.9113	0.1182	0.0906
SEASON*AGE*N	0.1812	0.8053	0.4067	0.7744	0.5742	0.2764	0.0388	0.0663
SP*SEASON*AGE	0.0071	0.0153	0.0001	0.5012	0.9295	0.3906	0.0284	0.1180
SP*SEASON*N	0.9725	0.8983	0.9165	0.8281	0.7584	0.5888	0.7121	0.5873
SP*AGE*N	0.1340	0.0414	0.4223	0.0919	0.4859	0.6356	0.6661	0.5350
SP*SEASON*AGE*N	0.4175	0.0347	0.0981	0.3962	0.5811	0.0470	0.2008	0.4477

† Concentration in total N.
‡ Concentration in total DM.

Table 4.4. Interaction effect of forage species and age of regrowth on herbage in-vitro digestible organic matter concentration in the early summer season. Data are means across N fertilizer rates (n=6).

Species	Age of regrowth (wk)			OPC †
	4	6	8	
	----------	g kg^{-1} OM	----------	
Limpograss	614 a‡	567 a	531 a	L
Bermudagrass	625 a	559 a	506 b	L
Bahiagrass	572 b	522 b	498 b	L,Q

† Orthogonal polynomial coefficients for ages of regrowth within species. L=linear and Q=quadratic.

‡ Forage species means within age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test.

Table 4.5. Interaction effect of forage species and age of regrowth on herbage in-vitro digestible organic matter concentration in the late summer season. Data are means across N fertilizer rates (n=6).

Species	Age of regrowth (wk)			OPC †
	4	6	8	
	----------	g kg^{-1} OM	----------	
Limpograss	576 a‡	571 a	523 a	L,Q
Bermudagrass	560 a	496 b	446 c	L
Bahiagrass	562 a	520 b	466 b	L

† Orthogonal polynomial coefficients for ages of regrowth within species. L=linear and Q=quadratic.

‡ Forage species means within age of regrowth of the late summer season not followed by the same letter are different (P≤0.05) by the LSD test.

Table 4.6. Interaction effect of age of regrowth and N fertilizer rate on in-vitro digestible organic matter concentration in the late summer season. Data are means across forage species (n=9).

	Age of regrowth (wk)			
N rate	4	6	8	OPC †
kg ha^{-1}	----------	g kg^{-1} OM	----------	
17	548	529	478	L,Q
50	584	529	479	L
P value ‡	0.0015	0.9696	0.9271	

† Orthogonal polynomial coefficients for ages of regrowth within N fertilizer rate. L=linear and Q=quadratic.

‡ Level of probability for effect of N fertilizer rate within age of regrowth on herbage in-vitro digestible organic matter concentration.

There was a species by season by age by N interaction for NDF (Table 4.3). Analyzing the data by season showed that in early summer there was an age by N interaction (Table 4.7) and a species by age interaction (Table 4.8). In the late-summer season there was a species by age by N interaction. Because there were no two-way interactions, the N main effect was not significant (P=0.4098), and the effects of species (P=0.0003) and age (P=0.0001) were the determinant factors, it was decided to present just these two main effects in late summer (Table 4.9).

The interaction of age and N rate in early summer reflected a lower NDF concentration with the lower N rate at 4 wk, but no response to N at ages 6 and 8 wk (Table 4.7). This decrease in NDF at 4 wk of regrowth using the high N fertilizer rate was also observed in the animal performance trial (Chapter 3). The interaction effect of forage species and age on NDF in early summer (Table 4.8) occurred

because bermudagrass and limpograss NDF concentration increased with age, but there was no effect of age on bahiagrass NDF concentration. Bahiagrass had or tended to have the lowest NDF concentration at all ages in early summer. In the late-summer season (Table 4.9) limpograss and bermudagrass had the highest NDF concentrations and both were greater than bahiagrass. The increase in NDF concentration from age 4 to 8 wk was described by including both linear and quadratic effects.

Table 4.7. Interaction effect of ages of regrowth and nitrogen fertilizer rates on neutral detergent fiber concentration in total DM in the early summer season. Data are means across forage species (n=9).

	Age of regrowth (wk)			
N rate	4	6	8	OPC †
kg ha^{-1}	----------	g kg^{-1} DM	----------	
17	747	754	773	L
50	727	756	761	L,Q
P value ‡	0.0203	0.6849	0.0651	

† Orthogonal polynomial coefficients for ages of regrowth within N fertilizer rate. L=linear and Q=quadratic.

‡ Level of probability for effect of N fertilizer rate within age of regrowth on herbage neutral detergent fiber concentration.

Table 4.8. Interaction effect of forage species and ages of regrowth on herbage neutral detergent fiber concentration in total DM in the early summer season. Data are means across N fertilizer rates (n=6).

Species	Age of regrowth (wk)			OPC †
	4	6	8	
	----------	g kg^{-1} DM	----------	
Limpograss	737 ab‡	776 a	774 a	L,Q
Bermudagrass	748 a	767 a	793 a	L
Bahiagrass	726 b	722 b	735 b	n.s.

† Orthogonal polynomial coefficients for ages of regrowth within species. L=linear and Q=quadratic.

‡ Forage species means within age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test.

Table 4.9. Main effects of forage species and ages of regrowth on herbage neutral detergent fiber concentration in total DM in the late summer season. Data are means across N fertilizer rates.

Species	Age of regrowth (wk)			Mean †
	4	6	8	
	----------	g kg^{-1} DM	----------	
Limpograss	729	736	784	750 a
Bermudagrass	741	750	779	757 a
Bahiagrass	711	716	744	724 b
Mean	727	734	768	L,Q ‡

† Forage species means not followed by the same letter are different (P≤0.05) by the LSD test (n=18).

‡ Orthogonal polynomial coefficients for ages of regrowth across forage species (n=18). L=linear and Q=quadratic.

There was a species by season by age interaction effect on leaf percentage (Table 4.3). When analyzed by season, there were species by age interactions in both seasons (Tables 4.10 and 4.11). Bahiagrass had the highest leaf percentage among species at all ages in both seasons. For all but the 4-wk regrowth in late summer limpograss had the lowest leaf percentage. High leaf percentage and low digestibility of bahiagrass, and low leaf percentage but high digestibility of limpograss go against conventional wisdom. The low IVDOM of bahiagrass has been associated with a higher presence and specific arrangement of sclerenchyma fibers ("girder" structure) in the leaves (Flores et al., 1993). High digestibility of limpograss stem and sheath have been reported previously (Holderbaum et al., 1992). Schank et al. (1973) found that high stem digestibility of tetraploid limpograsses like Floralta was associated with a smaller cross-sectional area occupied by vascular bundles than in less digestible diploids.

Herbage Nitrogen Fractions

There was a species by season interaction effect for herbage total N concentration and main effects of age and N fertilizer rates (Table 4.3). Nitrogen concentration increased with higher N fertilizer rate ($P=0.0001$), and there was a linear decrease in N concentration as age increased from 4 to 8 wk (Table 4.12). The species by season interaction occurred because during early summer bahiagrass had the highest N concentration (12.2 g kg^{-1}) (Table 4.13) while during late summer bermudagrass had the highest N concentration (15 g kg^{-1}). All species had higher N concentrations in the late summer season.

Table 4.10. Interaction effect of forage species and age of regrowth on leaf percentage in total dry matter in the early summer season. Data are means across N fertilizer rates (n=6).

Species	Age of regrowth (wk)			OPC †
	4	6	8	
	----------	%	----------	
Limpograss	29 c‡	22 c	22 c	L
Bermudagrass	46 b	39 b	41 b	L,Q
Bahiagrass	79 a	75 a	62 a	L

† Orthogonal polynomial coefficients for ages of regrowth within species. L=linear and Q=quadratic.

‡ Forage species means within age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test.

Table 4.11. Interaction effect of forage species and age of regrowth on leaf percentage in total dry matter in the late summer season. Data are means across N fertilizer rates (n=6).

Species	Age of regrowth (wk)			OPC †
	4	6	8	
	----------	%	----------	
Limpograss	45 b‡	35 c	27 c	L
Bermudagrass	46 b	39 b	37 b	L,Q
Bahiagrass	79 a	86 a	82 a	Q

† Orthogonal polynomial coefficients for ages of regrowth within species. L=linear and Q=quadratic.

‡ Forage species means within age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test.

Table 4.12. Main effect of N fertilizer and age of regrowth on herbage total N concentration. Data are means across forage species and seasons.

	Age of regrowth (wk)			
N rate	4	6	8	Mean †
kg ha^{-1}	----------	g kg^{-1} DM	----------	
17	12.3	10.4	8.0	10.3
50	14.1	11.5	9.5	11.7
OPC	13.3	11.0	8.8	L ‡

† N fertilizer rates across ages of regrowth are different (P=0.0001; n=54).
‡ Orthogonal polynomial coefficients for ages of regrowth across N fertilizer rates (n=36). L=linear.

Table 4.13. Interaction effect of forage species and season of the year (early and late summer) on herbage total N concentration. Data are means across ages of regrowth and N fertilizer rates (n=18).

	Summer season		
Species	Early	Late	P value †
	---------- g kg^{-1} DM ----------		
Limpograss	6.9 c‡	8.3 c	0.0091
Bermudagrass	10.6 b	14.5 a	0.0001
Bahiagrass	12.2 a	13.6 b	0.0106

† Level of probability for effect of season within forage species on herbage total N concentration.
‡ Forage species means within season not followed by the same letter are different (P≤0.05) by the LSD test.

The higher N concentration in bahiagrass compared with limpograss is typical of whole-canopy samples during summer in north Florida (Sollenberger et al., 1988 and 1989) and is comparable with the results reported by Brown and Pitman (1991) in south Florida. The N concentration for Tifton 85 bermudagrass is less than that reported by Hill et al. (1993), but lower N concentrations in the current study reflect longer regrowth periods, whole-canopy samples (10-cm stubble height) and relatively low N fertilizer rates. These N concentrations for Tifton 85 are comparable to those reported by Adjei et al. (1989) for 'Tifton 78' bermudagrass at the same ages of regrowth. The sharp decrease in N concentration of warm-season grasses with increasing age is well documented in the literature (Van Soest, 1982; Humphreys, 1987; Hodgson, 1990) and is normally associated with increased cell wall and lignin concentrations and a decrease in cell contents.

Quantifying herbage NDIN is important in understanding N fractionation in warm-season grasses. In this experiment only species and N fertilizer rate affected NDIN (Table 4.3), when the results were expressed as a proportion of total N. Approximately 40% of total N of these warm-season grasses was associated with the NDF (i.e, the cell wall) (Table 4.14). For comparison, NDIN concentrations from temperate grasses like timothy (*Phleum pratense* L.) or bromegrass (*Bromus inermis* Leyss.) were less than 160 g kg^{-1} of total N (Sanderson and Wedin, 1989). Limpograss and bahiagrass had the highest NDIN concentrations and bermudagrass the lowest. Increasing N fertilizer rate reduced the proportion of N associated with cell wall (Table 4.14).

The NDIN concentrations for limpograss in the current study (403 g kg^{-1} of total N) were less than those reported by Holderbaum (1989) (600 to 700 g kg^{-1}) using 110 kg N ha^{-1} and 5 wk of regrowth, and also less than the 544 g kg^{-1} reported by Brown and Pitman (1991). Bermudagrass NDIN averaged 374 g kg^{-1} of total N which was lower than the 580 g kg^{-1} reported by Brown et al. (1988) for four *Cynodon* genotypes. Sample processing in the current experiment may explain in part the lower NDIN concentrations observed. All samples were freeze-dried before grinding. According to Van Vuuren (1993) freeze-drying and subsequent grinding can rupture the cell wall, and free more protein and intact chloroplasts. This process maybe could make more N available to be digestible by the neutral detergent solution.

Table 4.14. Main effects of N fertilizer rate and forage species on herbage neutral detergent insoluble N concentration in total N. Data are means across seasons and ages of regrowth.

Species	N rate (kg ha^{-1})		Mean †
	17	50	
	g kg^{-1} of total N		
Limpograss	427	378	403 a
Bermudagrass	390	357	374 b
Bahiagrass	411	423	417 a
Mean ‡	409	386	

† Forage species main effect means not followed by the same letter are different ($P \leq 0.05$) by the LSD test (n=36).

‡ N fertilizer rates means across forage species are different ($P=0.0038$; n=54).

Herbage NDIN concentration in total DM was affected by a species by a four-way interaction (Table 4.3). When analyzed by season there was a species by N interaction in early summer (Table 4.15) and main effects of species and ages of regrowth in the late summer (Table 4.16). The interaction of species by N occurred in early summer because the effect of higher N rate was only significant for bahiagrass and not for the other two species. Within N fertilizer rate, bahiagrass had the highest NDIN concentration and limpograss the lowest. In the late-summer season there was a linear decline in NDIN in total DM from 4 to 8 wk of regrowth. There was no difference in NDIN concentration between bermudagrass and bahiagrass in late summer, but both had higher concentrations than limpograss.

Table 4.15. Interaction effect of forage species and N fertilizer rate on herbage neutral detergent insoluble N concentration in total DM in the early summer season. Data are means across ages of regrowth (n=9).

	N rate (kg ha^{-1})		
Species	17	50	P value †
	g kg^{-1} DM		
Limpograss	2.6 c‡	2.9 c	0.1076
Bermudagrass	4.0 b	3.8 b	0.5953
Bahiagrass	4.5 a	5.5 a	0.0007

† Level of probability for effect of N fertilizer rate within species on neutral detergent insoluble N.

‡ Forage species means within N fertilizer rate not followed by the same letter are different ($P \leq 0.05$) by the LSD test.

Table 4.16. Main effects of forage species and age of regrowth on herbage neutral detergent insoluble N concentration in total DM in the late summer season. Data are means across N fertilizer rates.

Species	Age of regrowth (wk)			Mean †
	4	6	8	
	----------	g kg^{-1} DM	----------	
Limpograss	3.6	3.2	2.5	3.1 b
Bermudagrass	6.2	5.7	4.5	5.5 a
Bahiagrass	6.6	5.9	4.8	5.8 a
Mean	5.5	4.9	3.9	L ‡

† Forage species means not followed by the same letter are different (P≤0.05) by the LSD test (n=18).

‡ Orthogonal polynomial coefficients for ages of regrowth across forage species (n=18). L=linear.

Whether it is a positive or negative characteristic to have more N associated with cell wall in warm-season grasses is a topic for discussion. Owens et al. (1991) pointed out that plant N inherently associated with cell wall is in closer proximity to digesting microbes than is N from supplements. This is important because proteolytic activity is about 75% particle associated and only 25% in the fluid phase (Brock et al., 1982). When ammonia is close to the major microscopic (interior of the cell wall) and macroscopic (the ruminal raft) sites of digestion, this can improve efficiency of cell wall-fermenting microbes (Owens et al., 1991). In both seasons, limpograss had the lowest NDIN in DM, primarily due to its low total N concentration.

Herbage ADIN is the N fraction insoluble in acid detergent solution and is largely unavailable to the animal (Goering et al., 1972). The sources of N bound to ADF are listed by Van Soest (1975) as the natural N in undamaged lignin, protein, or

amino acids bound by heating through the Maillard reaction, and the leather-like adducts between proteins and tannins. This fraction has been used to estimate indigestible N (Sniffen et al., 1992; Broderick, 1994) or fraction C in protein degradation models (Chalupa and Sniffen, 1991), a fraction that includes N that is neither degraded in the rumen nor digested in the intestine. Klopfenstein (1993) also considers ADIN as a reasonable measure of indigestible N. He suggested that ADIN is a very constant fraction in forages that normally ranges from 0.9 to 1.3% of DM in protein units (ADIN x 6.25). In the present experiment this generally was the case for limpograss and bahiagrass, but ADIN concentration in bermudagrass was not consistent across ages of regrowth (Tables 4.17 and 4.18) and seasons (Table 4.22), especially for bermudagrass.

When ADIN was expressed as a proportion of total N, it was affected by two, three-way interactions (season by age by N and species by season by age) (Table 4.3). When analyzed by season, there were species by age interactions for both seasons and a species by N rate interaction in the late-summer season. Bermudagrass and bahiagrass ADIN concentrations in total N increased linearly with increasing age in both seasons, but limpograss ADIN was not affected by age in early summer and increased only at 8 wk in late summer (Tables 4.17 and 4.18). Bahiagrass had or tended to have the lowest ADIN concentrations. These data were generally lower than means reported by Brown and Pitman (1991) for limpograss and bahiagrass (74 and 83 g kg^{-1} of total N, respectively) and lower than the 200 g kg^{-1} reported for

Table 4.17. Interaction effect of forage species and age of regrowth on herbage acid detergent insoluble N concentration in total N in the early summer season. Data are means across N fertilizer rates (n=6).

Species	Age of regrowth (wk)			OPC ‡
	4	6	8	
	----------	g kg^{-1} of total N	----------	
Limpograss	42 b†	51 b	49 b	n.s.
Bermudagrass	42 b	41 c	54 b	L
Bahiagrass	57 a	77 a	83 a	L

† Forage species means within age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test.
‡ Orthogonal polynomial coefficients for ages of regrowth within species. L=linear.

Table 4.18. Interaction effect of forage species and ages of regrowth on herbage acid detergent insoluble N concentration in total N in the late summer season. Data are means across N fertilizer rates (n=6).

Species	Age of regrowth (wk)			OPC †
	4	6	8	
	----------	g kg^{-1} of total N	----------	
Limpograss	42 b‡	41 b	56 c	L,Q
Bermudagrass	53 ab	67 a	69 b	L
Bahiagrass	56 a	74 a	89 a	L

† Orthogonal polynomial coefficients for ages of regrowth within species. L=linear and Q=quadratic.
‡ Forage species means within age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test.

Floralta limpograss by Holderbaum (1989). Tifton 85 bermudagrass generally had intermediate values for ADIN between bahiagrass and limpograss, but these values

were lower than 130 g kg^{-1} reported by Brown et al. (1988) for four *Cynodon* genotypes. Increasing N rates increased ADIN concentration in bahiagrass but had no effect in limpograss and bermudagrass (Table 4.19).

Table 4.19. Interaction effect of forage species and N fertilizer rate on herbage acid detergent insoluble N concentration in total N in the late summer season. Data are means across ages of regrowth (n=9).

	N rate (kg ha^{-1})		
Species	17	50	P value †
	g kg^{-1} of total N		
Limpograss	47 b‡	46 c	0.8375
Bermudagrass	60 a	66 b	0.1815
Bahiagrass	66 a	80 a	0.0081

† Level of probability for effect of N fertilizer rate within species on herbage acid detergent insoluble N.

‡ Forage species means within N fertilizer rate not followed by the same letter are different (P≤0.05) by the LSD test.

When expressed as a proportion of total DM, ADIN was affected by species by age of regrowth, species by N, and species by season interactions (Table 4.3). The species by age of regrowth interaction occurred because there was a linear decrease on ADIN concentration from age 4 to 8 wk in limpograss, but no effect of age on ADIN concentration in bermudagrass and bahiagrass (Table 4.20). Also, within ages of regrowth limpograss generally had the lowest ADIN concentration and bahiagrass the highest. The species by N interaction reflected a greater increase in ADIN concentration in bahiagrass with utilization of the higher N rate compared with the other two species (Table 4.21). The forage species by season interaction occurred because there was a great increase in bermudagrass ADIN concentration in late

summer, and smaller variations for limpograss and bahiagrass. Limpograss had always the lowest ADIN concentration in both seasons and bahiagrass the highest early in the summer, but not different from bermudagrass in the late summer season.

Table 4.20. Interaction effect of forage species and ages of regrowth on herbage acid detergent insoluble N concentration in total DM. Data are means across N fertilizer rates and seasons (n=12).

Species	Age of regrowth (wk)			OPC †
	4	6	8	
	----------	g kg^{-1} DM	----------	
Limpograss	0.43 b‡	0.32 c	0.30 c	L
Bermudagrass	0.71 a	0.70 b	0.66 b	n.s
Bahiagrass	0.85 a	1.00 a	0.93 a	n.s

† Orthogonal polynomial coefficients for ages of regrowth within species. L=linear.

‡ Forage species means within age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test.

According to Nolan (1993) the NPN fraction of immature forages may represent 300 g kg^{-1} of the total N present, and it is composed of nucleic acids, amides, amines, amino acids, and nitrate. Van Soest (1982) reported a broader range of NPN in grasses from 140 to 340 g kg^{-1} of total plant N. Reid (1994) suggested almost the same range for NPN fraction in forages (100 to 350 g kg^{-1} of total N).

Herbage NPN concentration in total CP was affected by a species by season by age interaction (Table 4.23). When analyzed by season there were main effects of

Table 4.21. Interaction effect of forage species and N fertilizer rate on herbage acid detergent insoluble N concentration in total DM. Data are means across ages of regrowth and seasons (n=18).

Species	N rate (kg ha^{-1})		P value †
	17	50	
	---------- g kg^{-1} DM ----------		
Limpograss	0.31 c‡	0.37 c	0.0132
Bermudagrass	0.64 b	0.74 b	0.0509
Bahiagrass	0.79 a	1.05 a	0.0001

† Level of probability for effect of N fertilizer rate within species on herbage acid detergent insoluble N..

‡ Forage species means within N fertilizer rate not followed by the same letter are different (P≤0.05) by the LSD test.

Table 4.22. Interaction effect of forage species and season of the year (early and late summer) on herbage acid detergent insoluble N in total DM. Data are means across ages of regrowth and N fertilizer rates (n=18).

Species	Summer season		P value †
	Early	Late	
	---------- g kg^{-1} DM ----------		
Limpograss	0.31 c‡	0.37 b	0.0134
Bermudagrass	0.48 b	0.90 a	0.0010
Bahiagrass	0.87 a	0.97 b	0.0036

† Level of probability for effect of season within species on herbage acid detergent insoluble N.

‡ Forage species means within season not followed by the same letter are different (P≤0.05) by the LSD test.

Table 4.23. Levels of probability for effects of forage species, season, age of regrowth (AGE), N fertilization (N), and their interactions on herbage nonprotein N (NPN), in-vitro readily soluble protein concentration (RSP) after 2 h of incubation, and in-vitro slowly soluble protein concentration (SSP) after 2 h of incubation.

EFFECT	NPN†	NPN‡	RSP†	RSP‡	SSP†	SSP‡
SPECIE (SP)	0.0001	0.0001	0.0001	0.0001	0.0001	0.0001
SEASON	0.0001	0.0001	0.6896	0.0002	0.0003	0.0001
SP*SEASON	0.0001	0.0001	0.2577	0.0014	0.0001	0.3302
AGE	0.0001	0.0023	0.1608	0.0002	0.0001	0.0001
N	0.3464	0.0007	0.4828	0.0216	0.5008	0.0001
SP*AGE	0.0001	0.8638	0.6443	0.2897	0.1538	0.6667
SEASON*AGE	0.0274	0.0088	0.0895	0.5390	0.9353	0.0866
AGE*N	0.5268	0.3877	0.1023	0.0795	0.5259	0.6782
SP*N	0.0083	0.0582	0.7372	0.4063	0.0676	0.2682
SEASON*N	0.8494	0.4081	0.7221	0.7030	0.5701	0.5348
SEASON*AGE*N	0.7865	0.4424	0.7987	0.9794	0.7614	0.9360
SP*SEASON*AGE	0.0192	0.1622	0.5351	0.4018	0.0425	0.4830
SP*SEASON*N	0.6746	0.7241	0.2465	0.6711	0.7490	0.8417
SP*AGE*N	0.4508	0.0688	0.0387	0.0098	0.4349	0.1549
SP*SEASON*AGE*N	0.8832	0.3720	0.9078	0.4929	0.9495	0.5501

† Concentration in total N.
‡ Concentration in total DM.

species and age in early summer and a species by age interaction in late summer. Bermudagrass had the highest NPN concentration in total N in early summer (159 g kg^{-1}) and 182 to 218 g kg^{-1} in late summer, followed by limpograss with 136 g kg^{-1} and 114 to 201 g kg^{-1}, respectively, and bahiagrass with 82 g kg^{-1} and 75 to 85 g kg^{-1}, respectively (Tables 4.24 and 4.25). These concentrations are lower than soluble N results reported for limpograss (390 g kg^{-1} of total N) and bahiagrass (352 g kg^{-1}) by Brown and Pitman (1991), but soluble N includes a small fraction of soluble true protein and the solubility methods utilized were different. Concentrations are similar to those reported by Holderbaum (1989). He estimated the NPN in Floralta limpograss to be 190 g kg^{-1} of total N. He suggested that NPN content remained relatively constant regardless of N concentration. Using different methods to measure N solubility in forages, Coto et al. (1990) reported a range of soluble N in bermudagrass from 390 to 460 g kg^{-1} of total N. Van Vuuren et al. (1991) suggested that the proportion of soluble N in fresh grass varies between 4 and 32% and increases with increasing N content.

Evaluating the N solubility of eight tropical grasses using the Burroughs mineral mixture, Aii and Stobbs (1980) reported a mean value of 338 g kg^{-1} of total N (range from 263 to 434 g kg^{-1}) with no effect of stage of growth (2, 4, and 6 wk). In the current study there was a linear increase in NPN concentration as a proportion of total N as age of regrowth increased in early summer. In late summer the same pattern was observed for limpograss and bermudagrass but there was no effect of age on NPN in bahiagrass.

Table 4.24. Main effects of forage species and ages of regrowth on herbage nonprotein N concentration in total protein in the early summer season. Data are means across N fertilizer rates.

Species	Age of regrowth (wk)			Mean †
	4	6	8	
	---	g kg^{-1} of total protein	---	
Limpograss	115	139	154	136 b
Bermudagrass	150	150	178	159 a
Bahiagrass	79	82	85	82 c
Mean	114	124	139	L ‡

† Forage species means not followed by the same letter are different (P≤0.05) by the LSD test (n=18).

‡ Orthogonal polynomial coefficients for ages of regrowth across forage species (n=18). L=linear.

Table 4.25. Interaction effect of forage species and ages of regrowth on herbage nonprotein N concentration in total protein in the late summer season. Data are means across N fertilizer rates (n=6).

Species	Age of regrowth (wk)			OPC †
	4	6	8	
	---	g kg^{-1} of total protein	---	
Limpograss	114 b ‡	153 b	201 a	L
Bermudagrass	182 a	221 a	218 a	L
Bahiagrass	75 c	83 c	85 b	n.s.

† Orthogonal polynomial coefficients for ages of regrowth within species. L=linear.

‡ Forage species means within age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test.

There were species by season and season by age interactions for herbage NPN in total DM (Table 4.23). The interaction between season and species (Table 4.26) occurred because NPN concentration in DM increased more from early to late summer for bermudagrass than in the other two grasses. Presented as a fraction of DM it becomes clear how low the NPN concentration in these warm-season grasses is (range from 0.9 to 3.0 g kg^{-1} DM). The season by age interaction occurred because NPN concentration decreased more between 4 and 6 wk in early summer but more between 6 and 8 wk in late summer (Table 4.27). For all ages of regrowth, NPN concentration was greatest in late summer.

Table 4.26. Interaction effect of forage species and season of the year (early and late summer) on herbage nonprotein N concentration in total DM. Data are means across ages of regrowth and N fertilizer rates (n=18).

	Summer season		
Species	Early	Late	P value †
	g kg^{-1} DM		
Limpograss	0.9 c ‡	1.2 b	0.0002
Bermudagrass	1.7 a	3.0 a	0.0003
Bahiagrass	1.0 b	1.1 b	0.0064

† Level of probability for effect of season within species on herbage nonprotein N concentration.

‡ Forage species means within season not followed by the same letter are different (P≤0.05) by the LSD test.

Table 4.27. Interaction effect of ages of regrowth and season of the year (early and late summer) on herbage nonprotein N concentration in the DM. Data are means across ages of regrowth and N fertilizer rates (n=18).

Age of regrowth	Summer season		P value †
	Early	Late	
wk	---------- g kg^{-1} DM ----------		
4	1.4	1.8	0.0414
6	1.1	1.9	0.0016
8	1.0	1.6	0.0023
OPC ‡	L,Q	Q	

† Level of probability for effect of season within age of regrowth on herbage nonprotein N concentration.

‡ Orthogonal polynomial coefficients for ages of regrowth within season. L=linear and Q=quadratic.

There was a species by age by N rate interaction effect for the in-vitro RSP after 2 h of incubation (Table 4.23) when expressed as a proportion of total N or total DM. As a proportion of N when analyzed by age of regrowth there were main effects of species and season at 6 and 8 wk of regrowth, and a species by N rate interaction at 4 wk (Table 4.28). The interaction of species by N rate occurred at 4-wk age of regrowth because higher N rates increased RSP of limpograss and bahiagrass but decreased RSP of bermudagrass. Bermudagrass had the highest fraction of RSP for most ages of regrowth, with exception of 6 wk when it was not different than bahiagrass. Limpograss generally had the lowest RSP.

Table 4.28. Interaction effect of forage species and N rate within the 4-wk age of regrowth and main effects of species within the 6- and 8-wk ages of regrowth on in-vitro readily soluble protein concentration in total protein determined after 2 h of incubation.

Species	Age of regrowth (wk)			
	4		6	8
	N rate (kg ha^{-1})			
	17	50		
	----------	g kg^{-1} of total protein		----------
Limpograss	56 b †	61 c	61 b ‡	68 b
Bermudagrass	114 a	106 a	111 a	119 a
Bahiagrass	58 b	90 b	91 a	85 b

† Forage species means of 4-wk regrowth within a N rate (SP x N; P=0.0236) not followed by the same letter are different (P≤0.05) by the LSD test (n=6).

‡ Forage species means within the 6- or 8-wk age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test (n=12).

When RSP was expressed as a proportion of total DM the analysis by age showed a species by N interaction at 4 wk, but only species main effects at ages 6 and 8 wk of regrowth. Species by N rate interaction at 4 wk occurred because increase in RSP with higher N rate was greater for bahiagrass than limpograss and bermudagrass. Bermudagrass had the highest RSP concentration in total DM at 8 wk but did not differ from bahiagrass at 6 wk. Limpograss had the lowest concentration at 6 and 8 wk (Table 4.29).

There was a species by season by age interaction for in vitro slowly soluble protein (SSP) concentration in total protein (Table 4.22). As described in the materials and methods section, SSP represents what was left after subtracting three

Table 4.29. Interaction effect of forage species and N rate within the 4-wk age of regrowth and main effects of species within the 6- and 8-wk ages of regrowth on in-vitro readily soluble protein concentration in total DM determined after 2 h of incubation.

Species	Age of regrowth (wk)			
	4		6	8
	N rate (kg ha^{-1})			
	17	50		
	g kg^{-1} DM			
Limpograss	0.54 c †	0.66 b	0.41 b ‡	0.34 c
Bermudagrass	1.53 a	1.68 a	1.41 a	1.26 a
Bahiagrass	0.81 b	1.43 a	1.20 a	0.86 b

† Forage species means of 4-wk regrowth within an N rate (SP x N; P=0.0127) not followed by the same letter are different (P≤0.05) by the LSD test (n=6).

‡ Forage species means within the 6- or 8-wk age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test (n=12).

fractions (NPN, RSP, and ADIN) from the total forage protein in the tube at the beginning of incubation. When SSP data were analyzed by season there were main effect forage species and age of regrowth in early summer and a species by age interaction in late summer. In both seasons, SSP concentration in total protein decreased linearly with increasing age (Tables 4.30 and 4.31). Also in both seasons, bahiagrass and limpograss had the highest SSP concentration and did not differ with the exception of the 8-wk age in late summer. Bermudagrass had always the lowest SSP concentration in early and late summer.

Table 4.30. Main effects of forage species and age of regrowth on herbage ruminal in-vitro slowly soluble protein concentration in total protein at 2 h of incubation in the early summer season. Data are means across N fertilizer rates and seasons.

Species	Age of regrowth (wk)			Mean †
	4	6	8	
	---	g kg^{-1} of total protein	---	
Limpograss	789	738	720	749 a
Bermudagrass	702	700	653	685 b
Bahiagrass	800	749	739	763 a
Mean	764	729	704	L ‡

† Forage species means not followed by the same letter are different (P≤0.05) by the LSD test (n=18).

‡ Orthogonal polynomial coefficients for ages of regrowth across forage species (n=18). L=linear.

Table 4.31. Interaction effect of forage species and age of regrowth on herbage ruminal in-vitro slowly soluble protein concentration in total protein at 2 h of incubation in the late summer season. Data are means across N fertilizer rates (n=6).

Species	Age of regrowth (wk)			OPC †
	4	6	8	
	---	g kg^{-1} of total protein	---	
Limpograss	780 a ‡	757 a	684 b	L
Bermudagrass	652 b	600 b	590 c	L
Bahiagrass	786 a	752 a	750 a	L

† Orthogonal polynomial coefficients for ages of regrowth within species. L=linear

‡ Forage species means within age of regrowth not followed by the same letter are different (P≤0.05) by the LSD test.

When expressed as a proportion of DM, SSP was not affected by any interaction but there were main effects of species, season, age, and N rate (Table 4.23). There was a linear decrease in SSP across forage species as age increased from 4 to 8 wk (Table 4.32). This probably reflects the reduction in N concentration with aging. Among species, bahiagrass had the highest SSP concentration because it had a similar total N concentration to bermudagrass, but had the lowest NPN and RSP fractions among all three grasses. Herbage SSP was greater in late than in early summer and increased with increasing N rate (Table 4.33)

Table 4.32. Main effects of forage species and ages of regrowth on herbage ruminal in-vitro slowly soluble protein concentration in total DM at 2 h of incubation. Data are means across N fertilizer rates and seasons.

Species	Age of regrowth (wk)			Mean
	4	6	8	
	----------	g kg^{-1} DM	----------	
Limpograss	8.0	5.4	3.8	5.7 c †
Bermudagrass	9.9	8.0	6.4	8.1 b
Bahiagrass	11.8	9.9	7.9	9.9 a
Mean	9.9	7.8	6.0	L ‡

† Forage species means not followed by the same letter are different (P≤0.05) by the LSD test (n=36).

‡ Orthogonal polynomial coefficients for ages of regrowth across forage species (n=36). L=linear.

Table 4.33. Main effects of seasons and N fertilizer rates on ruminal in-vitro slowly soluble protein concentration in total DM at 2 h of incubation. Data are means across ages of regrowth and forage species.

	Summer season		
N rate	Early	Late	Mean †
kg ha^{-1}	---------- g kg^{-1} DM ----------		
17	6.9	7.9	7.4
50	7.7	9.0	8.4
Mean ‡	7.3	8.5	

† N fertilizer rates across seasons are different (P=0.0001; n=54).
‡ Seasons across N fertilizer rates are different (P=0.0001; n=54).

Janicki and Stallings (1988) suggested that high correlations between in situ degradability and some in vitro measurements indicated that laboratory techniques can be utilized for estimating CP degradability of forages. They reported that for some hays NDIN had the greatest correlation with CP degradability (r=-0.83). A regression procedure using NDIN as the independent variable and the SSP fraction as the dependent variable was run using the 108 calculated values across species, seasons, ages, and N fertilizer rates. The generated equation SSP= 0.8679 + 1.621NDIN showed a good fit with an r^2 value of 0.85.

The SSP fraction was calculated as total N minus N degraded at 2 h (NPN + RSP) minus ADIN. The SSP fraction is considered to represent potentially degraded N because it was only exposed to the rumen inoculum during 2 h and because ADIN was subtracted from it. This fraction represents a large proportion of total herbage protein (600 to 800 g kg^{-1}) for these species. Unfortunately, without data on degradation rate and rate of passage it is not possible to establish what proportion of

SSP is slowly degradable protein or undegradable protein. The ranges for SSP in the present experiment were 684 to 789 g kg^{-1} of total protein for limpograss, 600 to 702 g kg^{-1} for bermudagrass, and 750 to 800 g kg^{-1} for bahiagrass. Brown and Pitman (1991) calculated a fraction that they called insoluble potentially degraded N (total N minus soluble N minus ADIN) and obtained values of 564 and 529 g kg^{-1} of total N for bahiagrass and limpograss, respectively. The calculation used to define SSP (SSP= Total N - [NPN + RSP] - ADIN) was basically the same as the one utilized by Brown and Pitman (1991). Because the total N and ADIN fractions were comparable in both trials, the big difference in the final results reflects the lower N degradability value of the term "NPN + RSP" compared with "Soluble N" utilized by Brown and Pitman (1991). These authors determined soluble N by incubating the samples in 25 ml of McDougall's buffer for 30 min and the forage residue was recovered on previously dried and weighed filter paper, then dried at 100°C for 24 h and weighed. Soluble N was calculated as the N loss occurring during the incubation as a percentage of the N originally incubated.

The ranges for the in-vitro RSP after 2 h of incubation can be considered very low. When the NPN and the RSP fractions are summed their ranges across all treatments were 150 to 300, 220 to 380, and 120 to 220 g kg^{-1} of total N, respectively, for limpograss, bermudagrass, and bahiagrass. These two fractions together are equivalent to NPN plus the rapidly degraded true protein fraction. The literature (Van Soest, 1982; Ellis, 1990; Hart and Leibholz, 1990; Klopfenstein, 1993; Merchen and Bourquin, 1994) indicates an extensive ruminal protein

degradation for fresh forage (especially grasses) in a range of 700 to 800 g kg^{-1} of total protein. A possible explanation for the low degradation of these warm-season grasses may be a lag time for initiation of N degradation in the in vitro process. A lag time for limpograss (no ammonia production at 4 and 8 h in an in vitro fermentation on McDougall's saliva) was reported by Brown and Pitman (1991). But they did not utilize inhibitors to avoid microbial utilization of protein degradation products. In the same experiment, bahiagrass did not show any lag period. After extensive in-vitro research with the IIV system, Broderick (1994) did not find improvement in incorporating a lag time into models describing protein degradation. Regarding this, he stated "Omission of a time-lag from most models describing ruminal protein degradation seems warranted."

To double check the results of the present experiment, 11 samples of the original material were sent to the U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI. The analysis requested was the inhibitor in vitro (IIV) procedure (Broderick, 1987) to estimate rate and extent of ruminal protein degradation. These analyses were run at two different incubation times (2 and 4 h) using duplicate samples and on two different incubation days. Even though the number of samples was limited, the results summarized in Table 4.34 show in general the same degradability trends observed in the results presented from analyses done in Gainesville.

Table 4.34. Degradation rate and estimated escape protein of 11 forage samples analyzed by the in-vitro inhibitor procedure at the U.S. Dairy Forage Research Center, USDA-ARS, Madison, WI.

Species	Degradation rate		Estimated escape	
	2 h	4 h	2 h	4 h
	h^{-1}		g kg^{-1} of total N	
Limpograss †	0.03	0.03	600	534
Bermudagrass ‡	0.05	0.03	430	480
Bahiagrass §	0.01	0.04	570	600

† n=3; ‡ n=5; § n=3

The slow degradation rate measured (0.01 to 0.04 h^{-1}) by the Wisconsin laboratory is in agreement with the low RSP and high SSP fractions observed in Gainesville. These results may confirm a slower protein degradability pattern in these grasses perhaps due to the higher proportion of protein associated with NDF. The estimated rumen undegraded protein calculated at the Dairy Forage Research Center is greater than that reported in the literature for temperate grasses and legumes and warm-season grasses. Most estimates of rumen undegradable protein are in the range of 100 to 400 g kg^{-1} of total protein [Van Soest, 1982 (fresh forages and silages); Ellis, 1990 (warm-season grasses); Van Straalen and Tamminga, 1990 (grass silage and hay) ; Merchen and Bourquin, 1994 (alfafa, clover, reygrass, pangola)]. What seems to be a problem with the high estimates of rumen undegraded protein calculated is that the degradation rate used (kd h^{-1}) is based on the fractions of protein undegraded at 0 and 2 h only. Maybe, as suggested by Sniffen et al. (1992), it should be necessary to calculate kds for each different fraction of the potentially

degraded protein (B1, B2, and B3). Also, the ruminal passage rate (kp) was assumed to be equal to 0.06 h^{-1} and a slower kp (maybe 0.04 h^{-1}) would fit better for beef cattle grazing these warm-season grasses (G.A. Broderick, 1995, personal communication).

Summary and Conclusions

The objectives of this experiment were to evaluate the influence of N fertilizer rate, age of regrowth, and season on concentration of various N fractions in three warm-season perennial grasses. The study also tested an in vitro analysis procedure to assess herbage N solubility and degradability. Limpograss was included in this study to aid in explaining responses observed in the animal performance trial reported in Chapter 3.

In general, Tifton-85 bermudagrass and Pensacola bahiagrass had the highest N concentrations, depending somewhat on the season, and Floralta limpograss always had the lowest total N concentration. Across species there was a linear decrease in the total N concentration as age increased from 4 to 8 wk.

Herbage in vitro digestible organic matter concentration is an important determinant of animal performance when forage is the only source of energy to grazing livestock. Forage species IVDOM were comparable at 4 wk of regrowth but with advancing maturity limpograss maintained higher levels of digestibility than the other grasses. Higher N fertilizer rate increased IVDOM at 4 wk of regrowth but had no effect at 6 or 8 wk.

Despite being somewhat lower in concentration than reported in other literature, the herbage NDIN fraction in this study composed almost half of total N in these warm-season grasses. Since the proteolytic activity in the rumen is about 75% particle associated (Brock et al., 1982), Owens et al. (1991) pointed out that this close proximity of N in the grasses to digesting microbes can improve efficiency of cell wall fermenting microbes. On the other hand, part of NDIN is composed by the ADIN fraction that is lignin bound and frequently unavailable to the animal.

Bahiagrass generally exhibited higher ADIN than limpograss and bermudagrass. Though ADIN concentrations generally were 80 g kg^{-1} of total N or less, this fraction composes a significant portion of an already limited nutrient in warm-season grasses.

The rationale for using the in vitro procedure proposed by Neutze et al. (1993), was to test an alternative approach to the in situ procedure (Broderick et al., 1988; Nocek, 1988; and Varel and Kreikemeier, 1995) for estimating forage rumen protein degradability. The in-vitro RSP concentrations after 2 h of incubation were very low ranging from 56 to 118 g kg^{-1} of total protein. Even when NPN, considered to be instantaneously degraded in the rumen was added to the RSP fraction the sum was in the range of only 130 to 340 g kg^{-1} of total protein. These results suggest that the amount of readily rumen available NPN and soluble true protein in these grasses may be too low for efficient microbial growth. Brown and Pitman (1991) also suggested that low quantities of soluble and degradable rumen N in limpograss and bahiagrass may affect microbial protein synthesis. The widespread

concept about temperate and warm-season forage grasses is that ruminal N degradation is in the range of 700 to 800 g kg^{-1} of total N (Van Soest, 1982; Ellis, 1990; Hart and Leibholz, 1990; Brown and Pitman, 1991; Klopfenstein, 1993; Merchen and Bourquin, 1994). Data from the current study indicated a high proportion of slowly rumen degraded protein in these grasses. These results are in agreement with Akin (1989) who reported that protein of warm-season grasses is generally more slowly degraded than that of cool-season grasses.

The primary objective of this experiment was to obtain more detailed information on N fractionation in warm-season grasses. The results should not be extrapolated beyond the species and treatments applied. Since no measure of rate of passage was obtained and there were no estimations of degradation rates for the different slowly degraded protein fractions (B1, B2, and B3), it is not possible to estimate forage rumen undegradable N or bypass N.

Further efforts are necessary to review the procedure in order to evaluate if particular characteristics of warm-season grasses such as very low N concentration and very high NDF and NDIN concentrations can interfere with the measurements of N degradability and fractionation. There is also a need to quantify what proportion of the SSP fraction is rumen undegradable N and rumen slowly degradable N.

In general, these warm-season grasses showed low total N concentration (especially limpograss) that decreased linearly with maturity, high NDF, and IVDOM that was not always positively related with leaf percentage. Despite having many physical and chemical similarities, these grasses had marked differences, particularly

in fractionation and degradation of N. Bermudagrass had the highest amount of readily available rumen N (NPN + RSP). On the other hand, protein in limpograss and bahiagrass was more slowly degradable. It is important to observe that more than 40% of total N was associated with NDF in limpograss and bahiagrass. The large proportion of N in NDF requires efficient microbial cellulolytic activity for the N to be made available. This can be accomplished by providing rumen degradable N (especially ammonia) and an energy source simultaneously. Limpograss had the lowest ADIN concentration, probably related in part to its lower total N concentration. Though present in small amounts ADIN is indigestible and unavailable and reduces the supply of an already limited nutrient in limpograss.

An overview of all N fraction means of limpograss by N fertilizer rate is presented at Table 4.35 in order to provide inputs to the grazing trial discussion.

Table. 4.35. Limpograss nitrogen fraction means by nitrogen fertilizer rate across seasons and ages of regrowth (n=18).

N fraction		N rate (kg ha^{-1})	
		17	50
N	(g kg^{-1} DM)	6.9 ± 2.5	8.3 ± 2.5
NPN	(g kg^{-1} of total N)	149 ± 33	143 ± 39
RSP	(g kg^{-1} of total protein)	66 ± 26	59 ± 16
NDIN	(g kg^{-1} of total N)	427 ± 88	378 ± 65
ADIN	(g kg^{-1} of total N)	47 ± 9	46 ± 8
SSP	(g kg^{-1} of total protein)	737 ± 49	752 ± 48

Particularly for limpograss, the low NPN and RSP concentrations measured are in agreement with the very low PUN values observed for the unsupplemented treatment (50NONE) in the animal performance trial. The ADG responses reported to pasture N fertilization and heifer supplementation with urea in Chapter 3 point to limitations in the quantity of soluble and degradable N at the ruminal level. The high SSP fraction of limpograss probably contains a significant proportion of rumen undegradable protein, but since the total N concentration is so low the amount of rumen undegraded protein reaching the intestines is small. Apparently the undegradable protein of limpograss did not meet heifer requirements in the grazing trial since a positive response to rumen undegradable protein supplementation was observed (at low N fertilizer rate). The characterization of N fractions of limpograss provides perspective to understand the effects of pasture N fertilization and protein supplementation on heifer performance.

APPENDIX

Table A.1. Summary of raw data from Chapter 3 for average daily gain (ADG, kg), carrying capacity (CC, heifer days per hectare), gain per hectare (GAIN, kg ha^{-1}), and plasma urea N (PUN, mg [100 $mL]^{-1}$).

REP	N	SUP	YEAR	ADG	CC	GAIN	PUN
1	50	1	1	0.116	512.2	59.4	3.48
2	50	1	1	-0.026	574.3	-15.1	2.75
1	50	2	1	0.311	525.3	163.5	13.90
2	50	2	1	0.446	581.7	259.3	16.80
1	50	3	1	0.599	566.9	339.4	17.32
2	50	3	1	0.496	588.8	291.9	14.93
1	150	1	1	0.417	638.8	126.4	7.58
2	150	1	1	0.198	639.5	266.5	9.01
1	150	2	1	0.451	709.6	233.9	15.27
2	150	2	1	0.330	586.1	264.3	18.27
1	150	3	1	0.469	621.1	291.6	17.35
2	150	3	1	0.417	747.2	311.3	17.52
1	50	1	2	0.127	623.2	79.3	6.23
2	50	1	2	0.032	720.3	23.3	4.29
1	50	2	2	0.500	560.0	279.9	13.35
2	50	2	2	0.375	704.5	264.1	16.10
1	50	3	2	0.507	604.5	306.4	19.27
2	50	3	2	0.627	620.2	389.0	15.87
1	150	1	2	0.412	677.7	279.2	9.13
2	150	1	2	0.396	647.2	256.1	11.05
1	150	2	2	0.477	587.1	279.9	17.60
2	150	2	2	0.312	658.5	205.7	17.62
1	150	3	2	0.592	701.8	415.8	16.08
2	150	3	2	0.419	782.0	327.6	18.77

Table A.2. Summary of raw data from Chapter 3 for in vitro organic matter digestibility (IVDOM, % OM), neutral detergent fiber (NDF, % DM), crude protein (CP, % DM), and digestible organic matter/ crude protein ratio (DOM/CP).

REP	N	SUP	YEAR	IVDOM	NDF	CP	DOM/CP
1	50	1	1	51.04	80.34	5.56	9.18
2	50	1	1	51.14	79.96	5.14	9.95
1	50	2	1	48.14	80.30	5.69	8.46
2	50	2	1	48.18	79.56	5.80	8.31
1	50	3	1	49.20	80.94	5.62	8.75
2	50	3	1	50.18	80.86	5.11	9.82
1	150	1	1	51.02	78.80	6.49	7.86
2	150	1	1	54.22	78.64	6.93	7.82
1	150	2	1	51.14	80.56	6.57	7.78
2	150	2	1	51.80	78.90	7.18	7.21
1	150	3	1	52.60	78.90	6.97	7.55
2	150	3	1	54.86	77.16	8.21	6.68
1	50	1	2	51.40	81.57	5.31	9.68
2	50	1	2	52.62	80.45	5.25	10.02
1	50	2	2	50.97	80.42	6.15	8.29
2	50	2	2	52.12	80.33	6.16	8.46
1	50	3	2	52.87	80.83	5.59	9.46
2	50	3	2	52.15	80.33	5.59	9.33
1	150	1	2	55.03	79.60	7.40	7.43
2	150	1	2	57.38	78.98	7.32	7.84
1	150	2	2	54.78	79.62	7.32	7.48
2	150	2	2	53.32	79.72	6.90	7.73
1	150	3	2	55.68	79.33	8.10	6.87
2	150	3	2	58.40	77.35	8.58	6.81

Table A.3. Summary of raw data from Chapter 3 for leaf blade crude protein (CPL, % DM), stem + sheath crude protein (CPS, % DM), leaf blade neutral detergent fiber (NDFL, % DM), stem + sheath neutral detergent fiber (NDFS, % DM), leaf blade in-vitro digestible organic matter (IVDOM LEAF, % OM), and stem + sheath in-vitro digestible organic matter (IVDOM STEM, % OM).

REP	N	SUP	YEAR	CPL	CPS	NDFL	NDFS	IVDOM LEAF	IVDOM STEM
1	50	1	1	9.3	3.5	73.7	84.0	47.9	45.4
2	50	1	1	9.0	3.0	72.1	84.3	50.3	43.8
1	50	2	1	10.3	4.0	76.3	84.1	47.7	46.6
2	50	2	1	9.2	3.6	76.3	85.2	47.1	44.3
1	50	3	1	9.6	4.0	74.7	85.0	46.2	46.7
2	50	3	1	9.2	3.0	74.2	84.6	49.2	46.3
1	15	1	1	10.9	3.1	72.4	85.1	51.1	45.1
2	15	1	1	9.8	3.6	74.4	85.8	49.5	47.9
1	15	2	1	10.8	4.4	72.3	82.8	50.2	48.5
2	15	2	1	11.0	5.3	76.2	83.2	49.4	50.9
1	15	3	1	10.0	4.2	74.4	83.0	49.4	47.5
2	15	3	1	11.9	5.7	72.9	78.6	55.6	54.9
1	50	1	2	10.0	4.1	76.4	83.0	49.7	50.9
2	50	1	2	9.5	3.9	75.1	82.7	51.4	50.6
1	50	2	2	9.8	4.0	76.7	82.5	49.9	52.0
2	50	2	2	10.2	4.3	76.2	82.6	50.4	54.5
1	50	3	2	10.1	4.3	77.8	83.0	52.8	52.8
2	50	3	2	10.6	4.3	74.3	81.4	51.3	53.3
1	15	1	2	11.3	5.5	76.4	78.8	49.3	55.4
2	15	1	2	12.4	5.7	74.9	80.4	56.0	58.1
1	15	2	2	12.0	5.5	75.0	80.1	51.8	55.7
2	15	2	2	11.5	5.3	76.4	81.5	51.1	55.1
1	15	3	2	12.6	5.9	77.5	80.1	52.4	58.0
2	15	3	2	13.5	6.9	76.4	78.3	59.8	62.4

Table A.4. Summary of raw data from Chapter 3 for percentage leaf blade in total dry matter percentage (LEAF, % DM), percentage stem + sheath in total dry matter (STEM, % DM), leaf blade/stem + sheath ratio (RATIO), and pregraze herbage mass (HM, kg ha^{-1}).

REP	N	SUP	YEAR	LEAF	STEM	RATIO	HM
1	50	1	1	21.75	78.25	0.29	3130
2	50	1	1	18.90	81.10	0.24	3315
1	50	2	1	23.00	77.00	0.31	2902
2	50	2	1	19.30	80.70	0.25	3472
1	50	3	1	19.55	80.45	0.25	2800
2	50	3	1	16.45	83.55	0.20	3878
1	150	1	1	19.00	81.00	0.24	3456
2	150	1	1	19.50	80.50	0.25	3900
1	150	2	1	23.15	76.85	0.35	3452
2	150	2	1	26.90	73.10	0.37	3294
1	150	3	1	28.40	71.60	0.40	2758
2	150	3	1	26.45	73.55	0.36	4120
1	50	1	2	28.17	71.83	0.39	4198
2	50	1	2	21.87	78.13	0.28	4049
1	50	2	2	26.00	74.00	0.35	3790
2	50	2	2	30.23	69.77	0.43	3365
1	50	3	2	26.77	73.23	0.37	3178
2	50	3	2	28.23	71.77	0.39	3612
1	150	1	2	31.07	68.93	0.45	3981
2	150	1	2	30.10	69.90	0.43	3558
1	150	2	2	25.07	74.93	0.33	3328
2	150	2	2	32.60	67.40	0.48	3478
1	150	3	2	33.13	66.87	0.50	3473
2	150	3	2	33.77	66.23	0.51	3953

Table A.5. Summary of raw data from Chapter 3 for herbage on offer (HO, kg DM ha^{-1} d^{-1}), average of kg of liveweight (AVKGLW, kg ha^{-1} d^{-1}), herbage allowance (HA, % liveweight), herbage consumed (HC, kg DM ha^{-1} d^{-1}), estimated intake (ESTIN, % liveweight).

REP	N	SUP	YEAR	HO	AVKGLW	HA	HC	ESTIN
1	50	1	1	390.0	2382	3.27	181.3	1.5
2	50	1	1	439.3	2671	3.29	183.2	1.4
1	50	2	1	426.7	2443	3.49	241.4	2.0
2	50	2	1	352.0	2706	2.60	164.8	1.2
1	50	3	1	335.8	2637	2.55	169.8	1.3
2	50	3	1	503.5	2738	3.68	216.3	1.6
1	150	1	1	419.7	2971	2.83	252.4	1.7
2	150	1	1	499.7	2975	3.36	271.7	1.8
1	150	2	1	418.1	2974	2.81	280.7	1.9
2	150	2	1	432.0	2726	3.10	253.6	1.7
1	150	3	1	347.9	2889	2.41	203.7	1.4
2	150	3	1	484.0	3475	2.79	319.5	1.8
1	50	1	2	555.1	2548	4.36	336.2	2.6
2	50	1	2	542.3	2873	3.77	234.5	1.6
1	50	2	2	508.7	2288	4.45	229.9	2.0
2	50	2	2	481.9	2883	3.34	148.3	1.0
1	50	3	2	430.3	2470	3.48	197.0	1.6
2	50	3	2	484.7	2535	3.82	126.4	1.0
1	150	1	2	539.1	2773	3.89	245.1	1.8
2	150	1	2	498.3	2646	3.77	153.3	1.2
1	150	2	2	448.1	2398	3.74	238.4	2.0
2	150	2	2	488.0	2693	3.62	250.2	1.9
1	150	3	2	450.9	2871	3.12	259.1	1.8
2	150	3	2	536.0	3203	3.32	181.9	1.1

Table A.6. Summary of raw data from Chapter 3 for average daily gain (ADG, kg), plasma urea N (PUN, mg [100 mL]$^{-1}$), crude protein (CP, % DM), in-vitro digestible organic matter (IVDOM, % OM), digestible organic matter/crude protein ratio (DOM/CP), and pregraze herbage mass (HM, kg ha^{-1}), by period (PER) in 1992.

PER	REP	N	SUP	ADG	PUN	CP	IVDOM	DOM/CP	HM
1	1	50	1	0.144	3.5	6.9	54.7	8.0	3418
1	2	50	1	0.113	3.1	5.7	53.4	9.4	4355
1	1	50	2	0.363	14.9	6.1	51.0	8.4	4355
1	2	50	2	0.287	16.6	5.9	50.8	8.6	4453
1	1	50	3	0.627	15.6	6.8	53.9	7.9	3963
1	2	50	3	0.582	15.9	5.0	52.2	10.5	5265
1	1	150	1	0.348	7.5	7.3	54.6	7.4	4895
1	2	150	1	0.106	10.2	7.5	55.8	7.4	4860
1	1	150	2	0.688	14.3	7.2	54.5	7.6	4395
1	2	150	2	0.454	17.0	8.3	56.2	6.8	4380
1	1	150	3	0.310	15.8	6.4	54.3	8.5	3978
1	2	150	3	0.461	16.6	7.7	56.5	7.3	6537
2	1	50	1	0.235	4.6	5.5	51.6	9.3	2740
2	2	50	1	0.162	2.3	4.1	51.0	12.4	3715
2	1	50	2	0.697	14.1	6.0	49.0	8.2	4055
2	2	50	2	0.478	16.1	7.0	48.9	7.0	2650
2	1	50	3	0.794	17.5	4.5	45.0	10.0	2725
2	2	50	3	0.332	14.6	6.1	49.3	8.1	3640
2	1	150	1	0.340	4.1	6.8	51.2	7.5	2310
2	2	150	1	0.794	10.3	8.2	55.3	6.7	4150
2	1	150	2	0.008	14.8	6.7	52.6	7.8	4125
2	2	150	2	0.462	17.2	7.0	53.0	7.5	2635
2	1	150	3	0.656	18.0	9.8	54.1	5.5	2340
2	2	150	3	0.632	18.4	10.0	56.6	5.7	3500
3	1	50	1	-0.032	2.3	4.3	47.2	11.0	3038
3	2	50	1	-0.364	2.9	5.2	49.0	9.5	2075
3	1	50	2	-0.130	12.8	5.2	44.9	8.7	2408
3	2	50	2	0.583	17.7	5.1	45.2	8.8	1478
3	1	50	3	0.373	18.9	5.0	46.7	9.3	1675
3	2	50	3	0.567	14.4	4.8	48.6	10.2	2610
3	1	150	1	-0.105	11.2	5.5	43.8	8.0	2590
3	2	150	1	0.373	7.1	5.7	52.1	9.1	2816
3	1	150	2	0.267	16.8	5.8	46.6	8.0	2173
3	2	150	2	0.437	20.1	6.2	46.9	7.6	2540
3	1	150	3	0.454	18.4	6.2	50.3	8.2	1748
3	2	150	3	0.154	17.6	7.8	53.5	6.8	2013

Table A.7. Summary of raw data from Chapter 3 for average daily gain (ADG, kg), plasma urea N (PUN, mg [100 mL]$^{-1}$), crude protein (CP, % DM), in-vitro digestible organic matter (IVDOM, % OM), digestible organic matter/crude protein ratio (DOM/CP), and pregraze herbage mass (HM, kg ha^{-1}), by period (PER) in 1993.

PER	REP	N	SUP	ADG	PUN	CP	IVDOM	DOM/CP	HM
1	1	50	1	-0.133	4.1	5.8	52.9	9.1	4775
1	2	50	1	0.055	3.2	5.6	53.8	9.6	5565
1	1	50	2	0.743	14.5	6.3	52.9	8.4	4268
1	2	50	2	0.180	16.6	5.9	52.9	9.0	4498
1	1	50	3	0.602	19.8	6.0	54.2	9.0	3928
1	2	50	3	0.782	16.3	5.7	53.4	9.4	3750
1	1	150	1	0.790	4.9	7.0	55.9	8.0	4988
1	2	150	1	0.102	11.0	7.6	58.0	7.6	3800
1	1	150	2	0.289	17.2	7.8	57.3	7.4	3750
1	2	150	2	0.188	16.1	7.1	54.6	7.7	4238
1	1	150	3	0.399	16.3	7.7	54.6	7.1	4338
1	2	150	3	0.180	21.1	8.5	59.8	7.0	5300
2	1	50	1	0.413	5.6	5.6	53.4	9.5	3705
2	2	50	1	0.267	4.8	5.7	53.4	9.4	2705
2	1	50	2	0.996	13.1	5.5	51.9	9.5	3005
2	2	50	2	0.907	13.9	6.2	55.9	9.0	2610
2	1	50	3	0.956	18.9	6.2	56.3	9.1	2790
2	2	50	3	0.705	15.5	6.5	55.8	8.6	2485
2	1	150	1	0.470	11.5	7.3	55.3	7.5	3435
2	2	150	1	0.850	11.5	7.5	58.0	7.7	2570
2	1	150	2	1.110	16.5	7.8	57.1	7.3	3235
2	2	150	2	0.640	18.0	8.2	58.0	7.1	2545
2	1	150	3	1.223	15.1	8.6	56.9	6.7	2880
2	2	150	3	0.859	18.0	9.8	62.5	6.4	2535
3	1	50	1	0.324	9.1	4.9	48.2	9.8	4670
3	2	50	1	0.008	4.9	4.9	51.4	10.5	3758
3	1	50	2	-0.316	12.6	6.0	48.0	8.0	4070
3	2	50	2	-0.340	17.9	5.7	49.3	8.6	3080
3	1	50	3	-0.162	19.1	5.3	49.0	9.3	3133
3	2	50	3	0.316	15.8	5.7	51.0	8.9	3603
3	1	150	1	0.049	12.9	7.6	52.7	6.9	3698
3	2	150	1	0.300	10.7	7.3	55.5	7.6	3855
3	1	150	2	0.073	19.2	7.1	52.4	7.3	3288
3	2	150	2	-0.024	18.8	6.2	50.7	8.1	3950
3	1	150	3	0.121	17.0	8.3	55.7	6.7	3313
3	2	150	3	0.356	17.3	8.5	56.6	6.7	3373

Table A.8. Summary of raw data from Chapter 4 (limpograss) for acid detergent fiber (ADF, % DM), neutral detergent fiber (NDF, % DM), in-vitro digestible organic matter (IVDOM, % OM), leaf percentage (LEAVES, % DM), and dry matter production (DM, kg).

REP	SEAS	AGE	N	ADF	NDF	IVDOM	LEAVES	DM
1	1	4	1	38.9	75.7	58.6	30.8	1560
2	1	4	1	36.4	73.3	62.3	24.2	1320
3	1	4	1	34.4	75.3	62.2	28.3	920
1	1	4	2	36.1	71.9	63.1	33.3	1320
2	1	4	2	37.0	73.3	61.5	26.1	2760
3	1	4	2	36.1	72.3	60.5	30.6	1440
1	1	6	1	37.4	76.7	57.4	19.1	4000
2	1	6	1	37.2	77.6	56.5	23.5	4140
3	1	6	1	34.6	78.1	57.3	28.6	2960
1	1	6	2	39.3	77.2	54.7	20.3	5180
2	1	6	2	36.3	79.0	56.7	21.2	4680
3	1	6	2	37.3	77.1	57.4	20.0	4360
1	1	8	1	37.8	78.1	53.3	21.8	4840
2	1	8	1	35.7	79.4	55.7	20.1	4380
3	1	8	1	35.6	77.9	53.5	20.4	4200
1	1	8	2	39.2	78.6	49.4	20.2	5140
2	1	8	2	35.2	75.5	53.1	25.0	3860
3	1	8	2	36.1	74.7	53.7	21.6	4180
1	2	4	1	36.9	73.6	56.1	49.3	1500
2	2	4	1	35.6	73.2	57.3	47.5	1600
3	2	4	1	35.2	73.8	55.9	41.8	1340
1	2	4	2	36.1	72.8	58.1	43.6	1820
2	2	4	2	34.9	70.2	61.9	48.8	1720
3	2	4	2	36.3	74.1	56.5	36.6	2520
1	2	6	1	36.1	73.6	54.5	31.2	3020
2	2	6	1	35.9	76.0	58.0	36.0	3000
3	2	6	1	34.4	71.7	56.1	39.5	2800
1	2	6	2	36.2	74.2	58.9	36.6	2980
2	2	6	2	35.3	72.2	58.4	34.9	2720
3	2	6	2	35.3	74.1	56.5	30.6	3940
1	2	8	1	35.7	78.1	51.7	23.3	4040
2	2	8	1	35.0	78.7	53.4	22.8	4180
3	2	8	1	35.7	78.0	53.9	28.6	3860
1	2	8	2	38.4	78.1	51.4	29.0	3620
2	2	8	2	36.3	79.0	51.1	28.5	3720
3	2	8	2	35.4	78.1	52.2	28.4	4280

Table A.9. Summary of raw data from Chapter 4 (bermudagrass) for acid detergent fiber (ADF, % DM), neutral detergent fiber (NDF, % DM), in-vitro digestible organic matter (IVDOM, % OM), leaf percentage (LEAVES, % DM), and dry matter production (DM, kg).

REP	SEAS	AGE	N	ADF	NDF	IVDOM	LEAVES	DM
1	1	4	1	36.0	75.2	61.7	46.0	2000
2	1	4	1	35.3	79.0	63.1	46.5	2020
3	1	4	1	36.2	74.4	61.6	50.0	1440
1	1	4	2	36.3	73.4	61.8	46.4	2760
2	1	4	2	35.7	73.3	65.1	46.8	1880
3	1	4	2	36.1	73.6	61.5	42.9	2800
1	1	6	1	38.4	76.0	57.4	36.8	4340
2	1	6	1	38.7	77.3	55.0	41.6	5660
3	1	6	1	37.1	76.2	57.3	38.5	4720
1	1	6	2	38.8	76.8	54.5	40.0	5460
2	1	6	2	39.8	77.8	55.3	37.8	6140
3	1	6	2	38.7	76.2	56.0	40.3	6240
1	1	8	1	39.7	79.2	49.0	41.2	3940
2	1	8	1	42.0	79.9	50.6	36.6	5920
3	1	8	1	39.1	78.6	49.9	44.6	3860
1	1	8	2	40.0	78.7	49.8	41.1	5180
2	1	8	2	42.1	79.4	48.0	41.2	6020
3	1	8	2	41.7	80.0	51.7	40.5	5500
1	2	4	1	38.3	76.5	54.9	45.7	1880
2	2	4	1	36.5	76.0	53.6	46.2	2080
3	2	4	1	38.7	73.6	52.9	46.0	1740
1	2	4	2	36.3	71.6	59.0	46.8	1880
2	2	4	2	37.0	74.1	55.9	45.2	2520
3	2	4	2	36.2	73.1	59.5	47.1	2080
1	2	6	1	35.9	68.3	53.4	40.8	980
2	2	6	1	37.5	74.0	51.5	41.7	1120
3	2	6	1	38.4	77.2	46.3	41.9	1240
1	2	6	2	40.4	76.7	50.3	38.3	1880
2	2	6	2	36.9	78.0	50.9	35.3	1360
3	2	6	2	39.2	76.1	45.2	37.9	1160
1	2	8	1	40.3	78.8	45.4	38.7	1860
2	2	8	1	40.4	78.6	41.5	39.6	1920
3	2	8	1	40.0	78.9	43.2	35.6	1800
1	2	8	2	39.3	77.6	43.7	33.3	2220
2	2	8	2	38.7	76.1	47.4	41.8	1960
3	2	8	2	40.0	77.0	46.5	34.7	1960

Table A.10. Summary of raw data from Chapter 4 (bahiagrass) for acid detergent fiber (ADF, % DM), neutral detergent fiber (NDF, % DM), in-vitro digestible organic matter (IVDOM, % OM), leaf percentage (LEAVES, % DM), and dry matter production (DM, kg).

REP	SEAS	AGE	N	ADF	NDF	IVDOM	LEAVES	DM
1	1	4	1	37.0	72.9	57.1	72.4	1520
2	1	4	1	37.7	73.0	56.6	83.3	1440
3	1	4	1	31.1	73.2	58.6	89.7	1160
1	1	4	2	37.8	71.9	55.7	77.8	1440
2	1	4	2	39.4	72.2	56.4	64.5	2480
3	1	4	2	36.6	72.4	59.0	83.2	1780
1	1	6	1	38.3	71.9	53.1	69.7	2480
2	1	6	1	38.8	72.7	53.2	73.5	2360
3	1	6	1	36.9	72.2	51.8	71.1	2560
1	1	6	2	36.9	72.4	50.2	71.6	2580
2	1	6	2	37.5	73.7	53.6	81.0	2600
3	1	6	2	36.4	70.1	51.0	80.0	2680
1	1	8	1	41.7	73.3	49.3	59.0	3380
2	1	8	1	39.6	73.5	52.5	53.8	3020
3	1	8	1	39.7	75.8	50.2	61.5	3080
1	1	8	2	39.4	72.2	50.2	65.7	4100
2	1	8	2	37.8	72.0	50.0	73.4	3420
3	1	8	2	38.5	74.2	51.2	60.6	3460
1	2	4	1	38.1	71.7	55.9	80.5	1640
2	2	4	1	38.5	71.6	51.7	75.0	1440
3	2	4	1	38.4	71.7	54.6	80.0	1400
1	2	4	2	38.1	71.0	59.8	76.2	1680
2	2	4	2	37.9	70.8	58.0	78.0	2280
3	2	4	2	38.7	70.0	57.3	85.7	2240
1	2	6	1	38.1	73.4	52.8	83.3	1440
2	2	6	1	40.3	72.4	52.1	88.5	2080
3	2	6	1	38.9	72.1	51.5	83.9	1740
1	2	6	2	37.1	70.9	52.3	89.7	1840
2	2	6	2	38.9	71.1	53.2	87.5	1920
3	2	6	2	36.2	69.7	50.3	84.5	2380
1	2	8	1	38.4	74.4	48.3	90.0	2480
2	2	8	1	39.6	74.2	46.1	87.2	2360
3	2	8	1	40.5	72.1	46.7	72.2	2760
1	2	8	2	39.1	72.9	46.1	81.1	3500
2	2	8	2	36.9	76.9	46.7	75.8	3060
3	2	8	2	41.8	75.7	45.8	85.5	3200

Table A.11. Summary of raw data from Chapter 4 (limpograss) for crude protein (CP, % DM); acid detergent insoluble N (ADIN, % total N); neutral detergent insoluble N (NDIN, % of total N), nonprotein N (NPN , % of total N), readily soluble protein (RSP, % CP); and slowly soluble protein (SSP, % CP).

REP	SEAS	AGE	N	CP	ADIN	NDIN	NPN	RSP	SSP
1	1	4	1	6.35	3.89	39.68	10.01	6.17	79.93
2	1	4	1	6.00	4.36	42.17	13.08	4.31	78.25
3	1	4	1	5.39	4.28	41.77	11.97	5.42	78.33
1	1	4	2	6.45	3.55	36.84	11.04	3.53	81.88
2	1	4	2	5.82	4.42	39.02	11.82	6.45	77.31
3	1	4	2	7.17	4.86	41.50	10.80	6.40	77.94
1	1	6	1	2.70	6.06	53.28	15.44	12.83	65.67
2	1	6	1	3.41	4.18	40.65	13.56	7.32	74.94
3	1	6	1	3.58	5.16	35.96	14.19	6.26	74.39
1	1	6	2	4.82	4.25	35.47	12.42	3.63	79.70
2	1	6	2	4.06	5.36	33.54	12.55	4.74	77.35
3	1	6	2	2.96	5.46	46.14	15.43	8.60	70.51
1	1	8	1	2.16	4.73	55.28	15.81	12.15	67.31
2	1	8	1	2.03	6.27	49.46	14.28	8.89	70.56
3	1	8	1	3.18	4.91	36.69	14.89	5.81	74.39
1	1	8	2	5.20	4.07	25.05	10.55	5.46	79.92
2	1	8	2	2.58	4.11	52.25	20.69	9.07	66.13
3	1	8	2	3.22	5.35	48.48	16.29	4.83	73.53
1	2	4	1	6.44	4.15	33.82	11.99	5.33	78.53
2	2	4	1	6.57	4.44	32.86	12.33	5.36	77.87
3	2	4	1	5.43	3.38	36.64	11.22	7.30	78.10
1	2	4	2	7.02	4.54	35.12	11.01	6.79	77.66
2	2	4	2	7.35	4.06	35.61	9.87	7.41	78.66
3	2	4	2	6.47	4.60	35.20	12.14	6.00	77.26
1	2	6	1	3.56	5.23	46.22	16.53	6.87	71.37
2	2	6	1	4.44	3.45	36.84	15.51	2.85	78.19
3	2	6	1	6.06	4.15	35.49	16.79	3.13	75.93
1	2	6	2	6.64	3.20	31.34	11.81	6.35	78.64
2	2	6	2	6.64	4.36	35.26	14.33	6.98	74.33
3	2	6	2	5.08	3.88	38.51	16.98	3.56	75.58
1	2	8	1	3.51	5.00	42.49	19.82	6.88	68.30
2	2	8	1	2.99	6.09	42.33	23.74	5.09	65.08
3	2	8	1	3.43	6.01	66.82	17.42	6.49	70.08
1	2	8	2	4.63	5.46	31.68	17.01	4.71	72.82
2	2	8	2	3.23	6.18	43.12	22.77	6.42	64.63
3	2	8	2	4.02	5.08	36.98	20.11	5.39	69.42

Table A.12. Summary of raw data from Chapter 4 (bermudagrass) for crude protein (CP, % DM); acid detergent insoluble N (ADIN, % of total N); neutral detergent insoluble N (NDIN, % of total N), nonprotein N (NPN , % of total N), readily soluble protein (RSP, % CP); and slowly soluble protein (SSP, % CP).

REP	SEAS	AGE	N	CP	ADIN	NDIN	NPN	RSP	SSP
1	1	4	1	7.80	3.69	39.20	12.39	12.61	71.31
2	1	4	1	7.27	4.19	42.08	14.55	7.67	73.59
3	1	4	1	7.86	4.99	39.54	12.96	12.26	69.79
1	1	4	2	9.21	4.57	36.59	15.10	9.39	70.94
2	1	4	2	8.79	3.04	34.56	16.81	10.02	70.13
3	1	4	2	8.82	4.76	32.84	17.89	11.84	65.51
1	1	6	1	6.20	4.50	41.17	14.62	12.19	68.69
2	1	6	1	6.03	3.77	28.57	13.67	10.77	71.79
3	1	6	1	6.43	3.61	37.59	14.39	10.93	71.07
1	1	6	2	6.60	4.85	37.99	15.49	10.81	68.85
2	1	6	2	5.50	3.72	41.49	15.57	9.64	71.07
3	1	6	2	6.21	4.27	35.53	16.15	11.08	68.50
1	1	8	1	4.19	4.37	43.68	17.64	9.51	68.48
2	1	8	1	5.83	6.22	39.71	16.98	11.55	65.25
3	1	8	1	6.05	5.37	37.68	17.50	11.41	65.72
1	1	8	2	5.40	4.98	26.42	15.85	13.80	65.37
2	1	8	2	6.23	6.76	39.70	20.08	12.31	60.85
3	1	8	2	4.51	4.82	29.25	18.87	10.39	65.92
1	2	4	1	8.35	5.72	42.90	15.49	12.83	65.96
2	2	4	1	8.43	3.60	40.38	14.55	11.59	70.26
3	2	4	1	10.42	6.39	40.19	22.02	11.44	60.15
1	2	4	2	12.71	5.25	35.17	19.10	9.30	66.35
2	2	4	2	9.20	4.88	37.84	18.62	11.15	65.35
3	2	4	2	11.01	5.91	37.67	19.14	11.76	63.19
1	2	6	1	11.94	5.70	37.19	21.38	12.00	60.92
2	2	6	1	9.28	6.87	39.18	21.95	11.57	59.61
3	2	6	1	8.79	6.38	40.12	21.04	12.52	60.06
1	2	6	2	7.58	6.68	36.61	21.86	6.87	64.59
2	2	6	2	10.89	6.26	33.38	23.67	12.94	57.13
3	2	6	2	8.56	8.01	38.95	22.69	11.79	57.51
1	2	8	1	5.93	6.78	40.08	17.92	12.74	62.56
2	2	8	1	6.68	5.90	31.51	23.97	10.87	59.26
3	2	8	1	7.17	6.74	41.82	19.92	12.48	60.86
1	2	8	2	7.40	7.80	37.39	21.52	10.96	59.72
2	2	8	2	9.27	7.00	37.44	23.85	14.63	54.52
3	2	8	2	9.44	7.20	33.51	23.66	12.14	57.00

Table A.13. Summary of raw data from Chapter 4 (bahiagrass) for crude protein (CP, % DM); acid detergent insoluble N (ADIN, % of total N); neutral detergent insoluble N (NDIN, % of total N), nonprotein N (NPN , % of total N), readily soluble protein (RSP, % CP); and slowly soluble protein (SSP, % CP).

REP	SEAS	Age	N	CP	ADIN	NDIN	NPN	RSP	SSP
1	1	4	1	8.78	5.79	43.62	8.21	3.46	82.54
2	1	4	1	7.94	5.73	41.37	10.27	4.82	79.18
3	1	4	1	8.26	4.87	42.08	7.58	6.30	81.25
1	1	4	2	9.71	6.45	44.20	5.74	9.83	77.98
2	1	4	2	9.45	6.19	40.38	8.32	7.60	77.89
3	1	4	2	9.33	5.14	45.21	7.26	6.57	81.03
1	1	6	1	7.27	6.77	39.45	8.55	11.78	72.90
2	1	6	1	7.56	7.02	40.09	7.74	7.87	77.37
3	1	6	1	6.62	6.62	40.26	9.10	6.81	77.47
1	1	6	2	8.38	8.94	42.04	9.40	11.09	70.57
2	1	6	2	7.59	8.37	43.89	7.92	6.23	77.48
3	1	6	2	9.43	8.57	41.70	6.76	10.93	73.74
1	1	8	1	5.68	8.50	28.24	8.73	12.33	70.44
2	1	8	1	4.72	8.13	49.65	7.52	14.67	69.68
3	1	8	1	7.05	9.28	31.24	8.67	4.61	77.44
1	1	8	2	6.32	6.43	37.96	9.78	7.36	76.43
2	1	8	2	6.95	9.60	43.38	7.75	7.76	74.89
3	1	8	2	6.12	7.75	40.77	8.28	9.31	74.66
1	2	4	1	9.45	4.83	40.08	8.33	5.97	80.87
2	2	4	1	8.43	5.22	40.01	8.99	7.96	77.83
3	2	4	1	9.55	4.98	42.16	7.89	6.18	80.95
1	2	4	2	11.17	6.15	45.48	5.96	10.60	77.29
2	2	4	2	9.58	5.89	45.39	7.29	8.92	77.90
3	2	4	2	10.23	6.45	40.34	6.63	10.19	76.73
1	2	6	1	7.71	7.64	45.73	9.15	4.88	78.33
2	2	6	1	7.90	6.98	43.99	6.37	11.19	75.46
3	2	6	1	8.01	6.94	41.72	8.17	11.09	73.80
1	2	6	2	9.13	6.59	44.12	8.33	9.37	75.71
2	2	6	2	8.72	8.78	40.15	8.77	9.88	72.57
3	2	6	2	10.17	7.49	40.03	9.05	8.20	75.26
1	2	8	1	6.27	6.52	44.27	6.98	11.09	75.41
2	2	8	1	7.36	8.95	45.08	7.87	11.08	72.10
3	2	8	1	6.13	7.59	40.73	9.19	6.44	76.78
1	2	8	2	7.86	10.63	40.08	8.78	8.27	72.32
2	2	8	2	7.21	8.58	39.07	9.98	4.34	77.10
3	2	8	2	7.60	11.19	46.31	8.46	4.35	76.00

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BIOGRAPHICAL SKETCH

Guilherme F. da Costa Lima was born in Recife, Pernambuco, Brazil, on 5 May 1954. He is the son of the late Oswaldo and Jacy Costa Lima. He has three brothers and two sisters.

He received the degree of Médico Veterinário from Universidade Federal Rural de Pernambuco in 1977. He received his M.S. in Animal Science from the same university in 1985.

He has been working since 1978 as a researcher in the area of forage management for EMBRAPA (Brazilian National Institute for Agricultural and Animal Husbandry Research) in the state of Rio Grande do Norte.

Guilherme enrolled at the University of Florida in the Fall of 1991 and is currently a candidate for the Doctor of Philosophy degree.

He is married to Susana (Cardoso) Costa Lima and they have three children: Manuela-19, Daniel-17, and Tiago-13.

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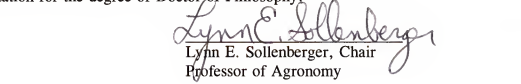

Lynn E. Sollenberger, Chair
Professor of Agronomy

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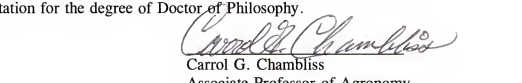

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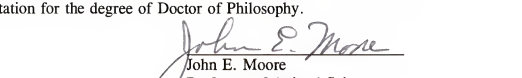

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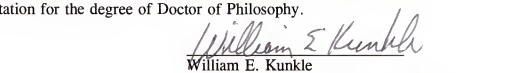

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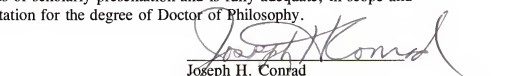

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate School and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

August, 1995

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